DOCTOR OF PHILOSOPHY

Promoting cellular adhesion to glass polyalkenoate cement by biopolymer addition

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Abdurahman Salem

Thesis submitted for the degree of Doctor of Philosophy

in Dentist

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DEDICATION

To

This thesis is dedicated to ***my father****, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to* ***my mother****, who taught me that even the largest task can be accomplished if it is done one step at a time. I also dedicate this dissertation to my uncle* ***Mr. Musa Salem*** *and my cousin* ***Professor Anwar Salem*** *for their huge support and encouragement. I will always appreciate all what they have done.*

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Acknowledgment is also due my brothers and sisters, for their overwhelming generosity, affectionate concern and incessant encouragement. Finally, heartfelt thanks go to my father and mother for their faith in me and to my friends, who have cheered, cajoled and supported me throughout.

DECLARATION

I declare that I am the author of this thesis and that I have consulted all the references cited. The work of which this thesis is a record has been accepted for a higher degree. This work has been carried out in the dental materials and Cell and Molecular Biology laboratories of Dundee Dental School, under the supervision of Professor R. G. Chadwick, Dr I. Ellis and Dr S. Jones.

Signature ……..

Date …….

Abdurahman Salem.

CERTIFICATE

I hereby certify that Abdurahman Salem has fulfilled the condition of Ordinance 39 of the University of Dundee and is qualified to submit this thesis for the degree of doctor of philosophy.

Professor R. G. Chadwick

Professor of operative Dentistry and Dental Material Science

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ABSTRACT

The American Academy of Operative Dentistry cites glass polyalkenoates (Glass Ionomer) as the material of choice to restore root surface caries lesions. Although apparently successful in the majority of cases it is not always possible to achieve a reliable seal from the oral environment when such lesion extends subgingivally. This is due to haemorrhage impeding placement and attachment of displaced gingival tissues.

This *in vitro* project comprises two inter related strands;

a) A postal survey of UK and Libyan dentists to ascertain their management strategies on root surface caries.
b) An investigation into the biocompatibility of two currently available glass polyalkenoates (GC Fuji VIII, ChemFil Superior) in manufactured form and in biologically modified forms to promote cellular attachment. Where results indicated this was appropriate to do so.

In addition the properties of surface hardness, strength [compressive, diametral, flexural (3 point and biaxial)], diametral fatigue strength and adhesive bond strength were determined prior to and following addition.

The results indicated that in Libya root caries was more often on interproximal surfaces than in the UK. Gingival bleeding was a common clinical problem at restoration placement. Additions of Type I Collagen and RGD to ChemFil Superior improved all physical properties measured except shear bond strength where no detriment was observed. GC Fuji VIII was shown by cell observation and viability tests not to be as biocompatible as ChemFil Superior.

Cell attachment to ChemFil Superior with the above addition was demonstrated by MTT. It is concluded that this approach has clinical potential to improve the restoration of root caries.

1 INTRODUCTION

Root surface caries is both prevalent and difficult to treat. Behavioural, cultural and age related changes play on an individual's susceptibility to this condition. Where prevention has failed operative intervention is required. In this process the gingival tissues become displaced and traumatised and the clinician is faced with a cavity extending downwards towards the root apex amidst blood from the gingival tissues. Under such circumstances a restoration of resin composite, due to appalling moisture control, is not going to work. Both dental amalgam and glass ionomer whatever offer some prospect of success. The latter also promises slow, fluoride release with the potential to seal the cavity by nature of its adhesive properties. Thus any residual caries may be deprived of its nutrient supply. This effective seal is however fragile for the cavity preparation often results in loss of tooth attachment to the gingival tissues. The incorporation of biopolymer additions to the material could offer cellular adhesion potential to reform this loss of attachment. To date little work has been carried out to address this possibility and this thesis therefore seeks to explore this possibility in some detail.

2 LITERATURE REVIEW

This literature review examines the origin and development of glass ionomer (glass polyalkenoate) cements and the treatment of root surface caries. It also explores manipulation of the behaviour of fibroblasts with emphasis upon achieving this to promote cellular attachment to dental restorative materials used to treat subgingival root caries.

2.1 GLASS IONOMER (GLASS POLYALKENOATE) CEMENTS

The glass-Ionomer cements were first developed at the laboratory of the government chemist by Wilson and Kent in the early 1970s (Wilson and Kent, 1972). They were derivatives of the earlier dental silicate and zinc polycarboxylate cements. The dental silicate cement is the result of a hardening reaction between an alumino-silicate glass (powder) and an aqueous solution of phosphoric acid (liquid) (Wilson and McLean, 1988). The resultant material has an appearance similar to tooth enamel, a high compressive strength, a coefficient of thermal expansion value that is equivalent to the tooth tissues and cariostatic properties as a consequence of the slow release of fluoride ions (Wilson and McLean, 1988).

The zinc polycarbcarboxylate cements consist of a zinc oxide powder and a poly-acrylic acid liquid (Smith, 1968). When the polyacrylic acid (liquid) is mixed with the zinc oxide (powder) the zinc ions react with the polyacid molecules linking them together in a gel like structure. The adhesion of the cement to the tooth is due to interaction of the polyacid with the calcium ions of the tooth structure (Smith, 1968).

The glass ionomer cements consist of an ion-leachable glass (powder) and a poly (alkenoic) acid (liquid).These components react together to form a tough cement

(Walls, 1986). The material combines the strength, rigidity and fluoride release of the dental silicates with the biocompatibility and adhesive characteristics of the poly acrylic acids and is an aesthetically acceptable adhesive restorative material (McLean and Wilson, 1977a). Since such developments a number of modifications and changes have been carried out to both the glass ionomer powder and liquid with a view to improving the properties of these materials (Moshaverinia et al., 2012).

2.1.1 TYPES OF GLASS IONOMER CEMENTS

The term glass ionomer refers to a material that involves a considerable acid–base reaction as part of its setting process, where the acid is a water-soluble polymer and the base is a special glass (McLean et al., 1994). Two types of glass ionomer cement presentation exist; the conventional, prepared from a glass powder and a concentrated solution of a polyalkonic acid (Wilson and Kent 1973), and the resin modified glass ionomer. In recent years such materials have been renamed glass polyalkenoate cements by the International Standards Organisation (ISO), a term that better reflects their setting chemistry. Both terms glass ionomer and glass polyalkenoate are used in this thesis.

2.1.1.1 *CONVENTIONAL GLASS IONOMERS CEMENT*

The conventional glass ionomer cement is considered to be the simplest form of glass ionomer cement; it is formed by an acid-base reaction between an ion leachable glass and a poly acid (Culbertson, 2001). Its setting reaction includes the neutralization of acid groups of a water-soluble acid polymer by a chemically basic powder. The powder is a special calcium alumina-silicate glass that also contains fluoride. Incorporation of this last element is an important feature of this material because it enables it to release clinically useful quantities of fluoride to theoretically prevent the occurrence of recurrent caries around restorations (Swartz et al., 1984).

2.1.1.2 *RESIN MODIFIED GLASS IONOMER CEMENT (RMGI)*

This type of glass ionomer cement is also known as a visible light cured (VLC) glass ionomer cement. It is a hybrid type of material, which cures by both a free-radical resin polymerisation and an acid base neutralisation reaction. The resin modified glass ionomer cements (RMGI) therefore set partially via an acid-base reaction and partially via a photochemical (visible light) or redox polymerisation process (Culbertson, 2001).

2.1.1.3 *MISNAMED GLASS IONOMERS CEMENT*

Other materials marketed by some companies as ”light-cured glass ionomer”, are basically resin composites, even though these materials contain the fluoroalumioslicate glass of conventional glass ionomer cement. However, these materials do not have the good adhesive properties of glass ionomers and release

lesser quantities of fluoride. They also tend to contract during setting (Nicholson, 1997).

2.1.2 COMPOSITION OF GLASS IONOMER

The compositions of commercial glass ionomer cements differ and are complex. No two commercial products of glass ionomer are chemically identical. All glass ionomer products however share certain common elements (Wilson and McLean, 1988). The original glass ionomer cements were formed by a reaction between polymers of polyacrylic acid with a powder that contained a calcium fluoro-alumino-silicate glass (Kent et al., 1973). Since then, in an endeavour to improve their properties, lots of changes and modifications to both the powder and liquid components of the original glass ionomer cement have been made. These have also been carried out to circumvent patented technology and bring to the market products of rival manufacturers. A consequence of this is that there are considerable differences in the composition and properties of commercial types of glass ionomer materials to cater for different applications and uses (Moshaverinia et al., 2012).

2.1.2.1 *GLASS COMPOSITION*

The original ion leachable glasses were based on a calcium alumino-silicate glass with high fluoride contents known as G200 (Table2-1) (Wilson and McLean, 1988). This was the culmination of much research effort that resulted in a glass optimised for chemical reaction that yielded the most desirable physical properties.

Table 2-1 The composition of the original glass ionomer (G-200) (modified from Wilson and Mclean (1988)).

Component	Percent (%)
SiO_2	30.1
Al_2O_3	19.9
AlF_3	2.6
CaF_2	34.5
NaF_2	3.7

Fluoride is an important component of ionomer glass; at manufacture it decreases the temperature of glass fusion, improves working properties of the cement paste and increases the strength of the set cement. Moreover, it has some therapeutic effects on the restored tooth (Wilson and McLean, 1988) though this last property is disputed by some (Mickenautsch et al., 2011).

The inclusion of Silica (SiO_2), Alumina (Al_2O_3), and Calcium Fluoride or Fluorite (CaF_2) enables the formation of a glass that is a fusion of these compounds. They produce a glass suitable for reaction with a polyacid with subsequent formation of a dental cement (Wilson and McLean, 1988).

The glasses are formed by fusing the raw components at high temperatures between 1100 °C and 1500 °C (the fusing temperature is depended upon the materials used to make the fusion mixture) in a sillamite crucible, then pouring the resultant molten glass frit onto a metal plate, that is quenched in cold water. One solidified the glass is then ground to give an ultimate particle size of no more than

50 μm in diameter for restorative cements and 20 μm for luting cements (Wilson and McLean, 1988).

The physical properties of the set glass ionomer may be improved by adding some non-matrix-forming inclusions into the glass (Walls, 1986). Metallic inclusions fused to glass offered potential to improve the physical properties of the set cement, for example the inclusion of crystallites of Corundum, Rutile, Aluminium Titanate and Baddeleyite into the glass were found to improve the flexural strength of the set cement (Walls, 1986). These found application in commercial products such as the cermets when gold and silver were used. It would however be true to say that these materials have found limited clinical application by clinicians in the UK (McLean, 1992).

2.1.2.2 *LIQUID COMPOSITION*

In the early glass ionomer cement, a 50 % aqueous solution of polyacrylic acid was used to react with the glass powder (Walls, 1986). This solution was unstable and prone to gelation during storage, due to a slow increase in the number of hydrogen bonds within the solution (Crisp et al., 1975, Walls, 1986). Some different types of polyacids used to form glass ionomer cement are shown in Figure 2-1. The functionality and strength of these poly acids differs depending on the acid structure and the molecular weight and concentration of the acid (Lohbauer, 2009). The poly maleic acid has double the number of carboxyl groups than the polyacrylic acid and is a stronger acid (Wilson and McLean, 1988). It is more active and therefore needs less reactive glasses than those used in combination with polyacrylic acid in order to form a set material (Wilson and McLean, 1988).

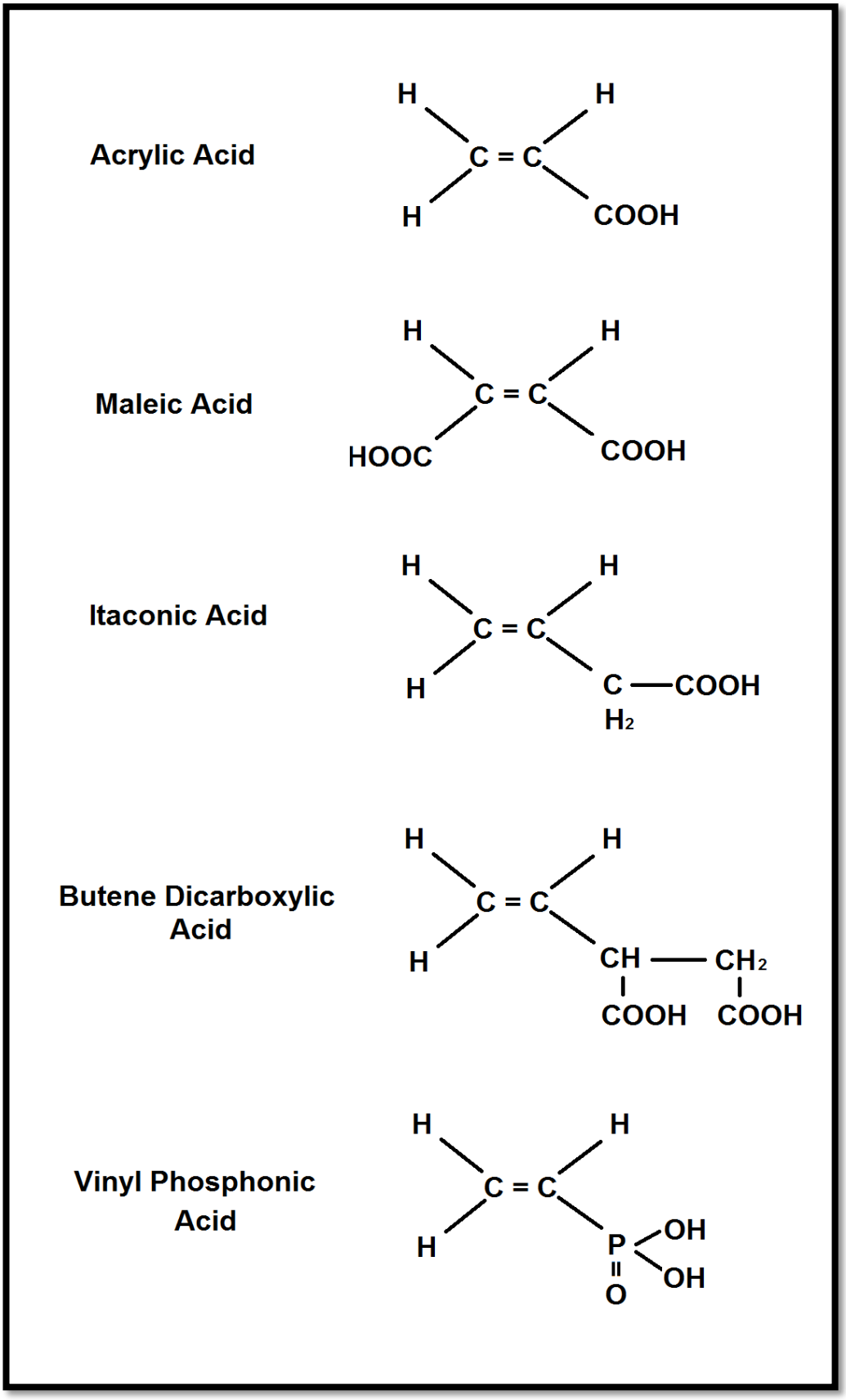


Figure 2-1 Polycarboxylic acids used to form Glass ionomer cements (Lohbauer, 2009)

The polyacid can be presented either in the form of a 40% to 50% aqueous solution of the poly acid or as a freeze-dried poly acid mixed with glass powder. In the latter case the setting reaction is started by mixing with either water or an aqueous solution of tartaric acid (McLean et al., 1984).

The addition of small amounts of optically active isomers of tartaric acid increases the rate of the setting reaction and also the ultimate compressive and tensile strength of the glass ionomer cements. Such an addition however, has no significant effect on the working time of the material (Wilson et al., 1976).

2.1.3 THE SETTING REACTION

The setting reaction of the glass ionomer cements is complex and differs according to the composition of the material (Culbertson, 2001). The setting reaction of glass ionomers is principally an acid-base reaction between the poly (alkenoic) acid and the glass particles (Wilson and McLean, 1988).

Three overlapping phases are distinguishable in the setting reaction of glass ionomers (Figure 2-2) (Crisp and Wilson, 1974b);

1- Initial poly-acid attack upon the outer layer of the glass particles in which ion leaching occurs.

2- Precipitation process and salt hydrogel formation.

3- Reaction and diffusion processes that continue for several months.

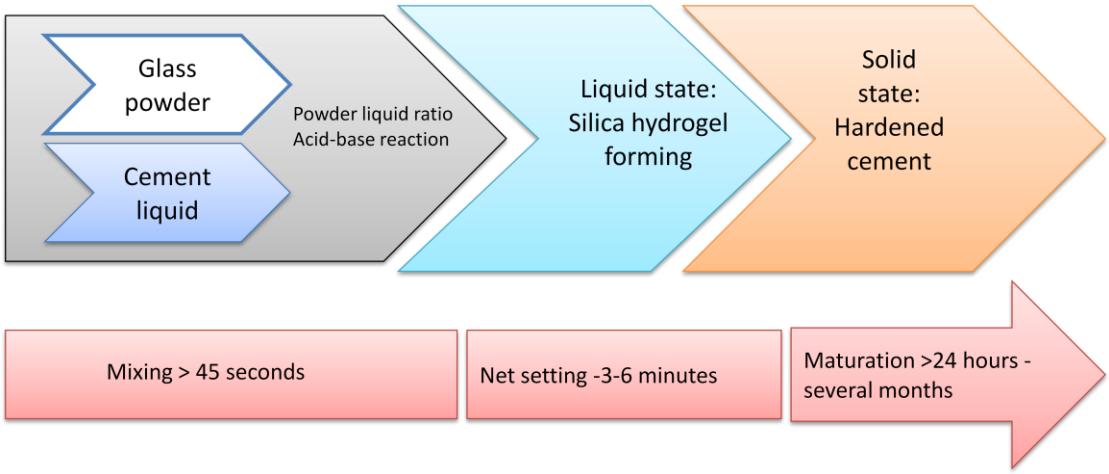


Figure 2-2 Glass ionomer setting reaction stages

The setting reaction starts by the attack of the poly acid upon the surface layer of the glass particles in the presence of water, this releases cations, (mainly calcium, aluminium, and fluoride ions from the glass) to form a silaceous hydrogel (Culbertson, 2001).

The liberation of metallic ions is facilitated by the presence of chelating agents such as D-Tartaric acid (Wilson et al., 1976 , Prosser et al., 1982).

An increase in pH and concentration of soluble ions derived from the glass demonstrate that the initial reaction is an acid base one where the powder acts as a proton acceptor and the liquid a proton donor. The essential change is that hydrogen ions in the liquid are replaced by metallic ions (Crisp et al., 1974).

In the early stages of the reaction the calcium salt (calcium poly-acid) alone is formed (Crisp et al., 1974). This corresponds to gelation and the initial set of the material. Final hardening occurs as the aluminium salt (aluminium poly-acid) is formed (Crisp et al., 1974)

The hydrogel may be considered as the materials binding matrix. Inter and intramolecular salt-bridges are formed within the poly acid by their release the calcium and aluminium ions, form a hard cross linked, ceramic like cement, with some molecular structures retained in the matrix (Figure 2-3) (Culbertson, 2001).

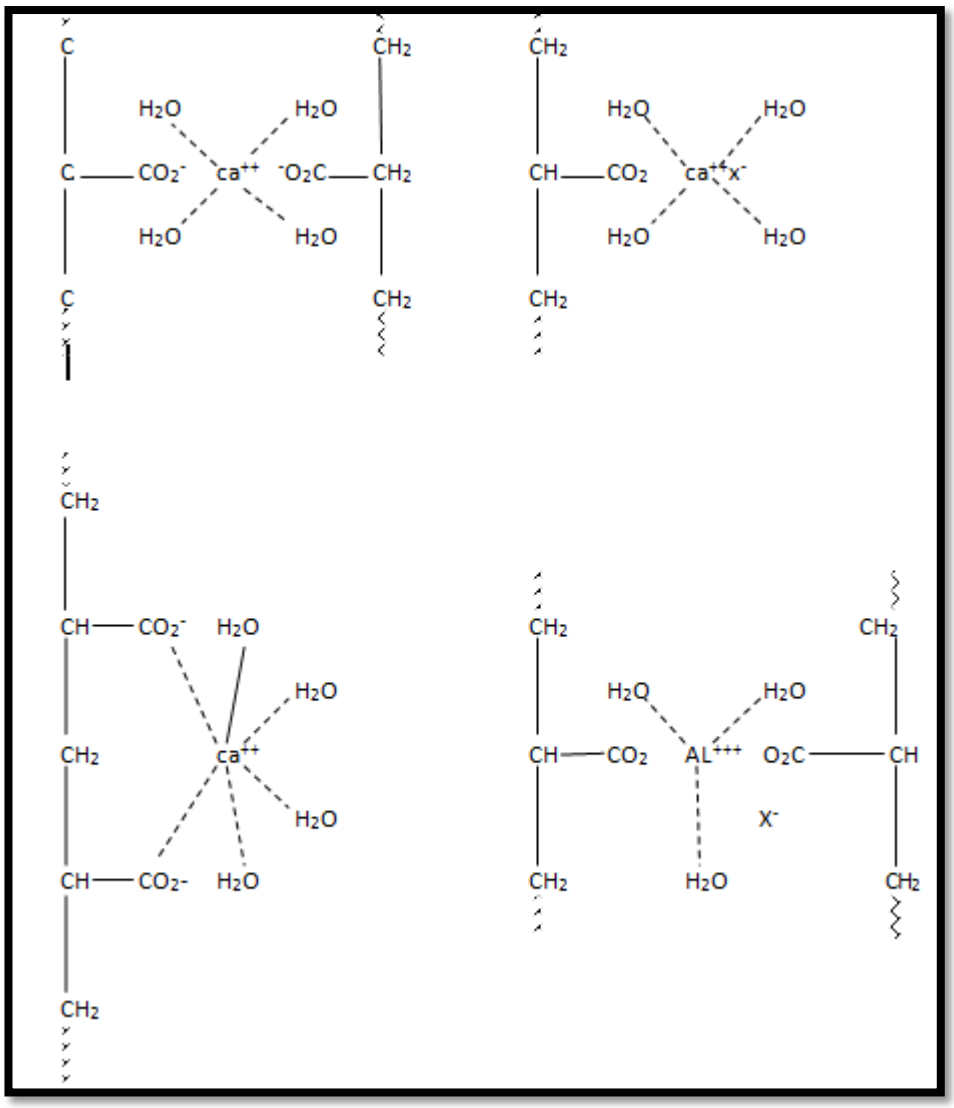


Figure 2-3 Inter-and intramolecular calcium or aluminium carboxylates (salt-bridges) in set glass ionomer cements. Where X represents OH^- or F^- (modified from Culbertson (2001)).

The cement undergoes further hardening in the first 24 hours following mixing and beyond. During this time translucency develops as the chemical reactions continue, the ratio of bound and unbound water increases, and the mechanical properties (compressive strength) increase gradually until their maximum values are eventually reached (Wilson and McLean, 1988).

There are a number of factors affecting the setting reaction and the final strength of the glass ionomer cement (Wilson and McLean, 1988). These factors include; the type and composition of polymers or copolymers, the presence or absence of tartaric acid or chelating agents, the composition of the glass powder and the powder liquid ratio used to mix the material (Smith, 1990) .

The final set structure of the glass ionomer cement consists of the original glass particles surrounded by a siliceous hydrogel that is bounded by a matrix phase consisting of hydrated fluoridated calcium and aluminium poly acrylates (Smith, 1990) (Figure 2-4).

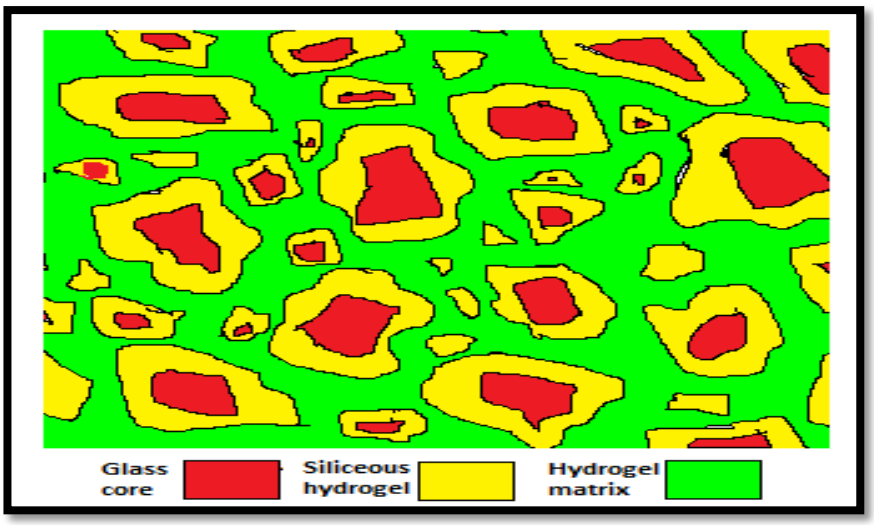


Figure 2-4 The fully hardened glass ionomer cement the matrix phase and the structure of the filler (glass core attached by a silceous hydrogel) smaller particles are completely degraded to siliceous hydrogel. (Modified from Wilson and McLean (1988)).

In the laboratory raising the temperature of conventional glass ionomer to 333-343 degrees Kelvin almost sets such materials on demand and improves early compressive strength (Algera et al., 2006).

2.1.4 PROPERTIES OF GLASS IONOMER

Conventional glass ionomer cements are clinically attractive materials and have unique properties that make them useful dental restorative materials. They set quickly in the oral environment in three to eight minutes; their translucency matches those of the tooth enamel especially in the case of recent commercial materials. The glass ionomer cements however are slightly brittle materials though they deform a little under load. They display high compressive strengths but slightly weak flexural strengths; compared to other dental cements they have a higher resistance to acid erosion and have the ability to form permanent adhesive bonds to dental enamel and dentine irrespective of the type of glass ionomer. They also release fluoride over prolonged periods of time (Wilson and McLean, 1988).

2.1.4.1 *THE MECHANICAL PROPERTIES*

Glass ionomer cements are moderately hard brittle materials, that display an ability to adhere to mineralized tooth structures (Walls, 1986). *In vitro* flexural or compressive strength testing of glass ionomer cements has been revealed to closely model the clinical loading situation (Lohbauer, 2009). Under compressive stress the glass ionomer cements (ASPA) (167 N mm^{-2}) were considerably stronger than polycarboxylate cements (60-85 N mm^{-2}) but usually weaker than dental silicate cements (250 N mm^{-2}) (Crisp et al., 1975). The tensile strength of early

glass ionomer cements (ASPA) (10-17 N mm^{-2}) is higher than the tensile strength of polycarboxylate cements (6-12 Nmm^{-2}) but is almost in the same range as the tensile strength of silicate cements (15 Nmm^{-2}) (Crisp et al., 1975).

The alteration in composition of the glass powder, by the incorporation of some metallic particles has been found to have some effect on the mechanical properties of glass ionomer cement (Walls, 1986). Williams et al (1992) compared the *in vitro* mechanical properties (compressive and diametral strengths in addition to tensile strength using the four point test) of metal reinforced and non–metal reinforced glass ionomer cements. It was found that the reinforced materials displayed significantly higher strengths than all the other materials though there was less difference in compressive strength. Table 2-2 summarises the mechanical properties of ten commercial glass ionomer cements (Xie et al., 2000).

Table 2-2 The mechanical properties of ten commercial glass ionomer cements (Xie et al., 2000).

Materials	FS (MPa)	CS (MPa)	DTS (MPa)
Ketac-bond	11.1 (1.1)	222.7 (7.3)	20.0 (0.8)
α-Silver	31.4 (3.4)	176.0 (6.5)	18.7 (0.9)
α-Fil	26.8 (3.1)	196.5 (8.3)	18.2 (1.0)
Ketac-Silver	22.9 (1.9)	211.8 (3.2)	22.1 (1.3)
Ketac-Fil	22.6 (2.5)	251.2 (10.0)	25.2 (0.6)
Ketac-Molar	21.2 (3.1)	301.3 (10.1)	23.8 (1.1)
Fuji II	26.1 (3.9)	202.0 (10.0)	20.1 (0.6)
Vitremer (Tricure)	82.1 (3.3)	265.3 (7.5)	47.5 (0.9)
Fuji II LC (Improved)	71.1 (3.6)	306.2 (6.8)	44.4 (1.1)
Photac-Fil	74.4 (5.1)	243 (7.6)	37.9 (1.7)

Mechanical properties (flexural strength (FS), compressive strength (CS), and diametral tensile strength (DTS))

Variations in powder liquid ratio, the concentration and molecular weight of the poly acid, and the proportion of tartaric acid, together with the amount of hydration and proportions of loose and tight water of the cement have been shown to have some effect on the mechanical properties of the glass ionomer cements (Walls, 1986). The effect of these variations on the properties of glass ionomer cements properties are summarised in Table 2-3.

Table 2-3 The effect of component variation on the glass ionomer cements properties (modified from Walls (1986)).

Variable	Working time	Setting time	Early hardness	Compressive strength	Tensile strength	24-hour water Leachable material
Increased P : L ratio	Decreased	Decreased	Increased	Increased	_____	Decreased
<38% Poly-acid concentration	Unaffected	Decreased with increasing concentration	_____	Increased with increasing concentration	Increased with increasing concentration	Decreased with increasing concentration
Increased molecular Weight of the poly acid	Decreased	Decreased	_____	Increased	Increased	_____

P=powder, L=liquid.

More recently the effects upon compressive strength of adding both boric and phosphoric acids to conventional glass polyalkenoate have been investigated (Prentice et al., 2006). The addition of 1% boric acid decreased the compressive strength whereas the incorporation, of up to 2% of phosphoric acid resulted in an increase in this property. This was attributed to increased cross linking of the acid chains with aluminium.

Moshaverinia et al. (2008b) demonstrated that copolymers of acrylic acid and N-Vinylpyrrolidone, with side chains of itaconic acid, improved the physical properties (compressive and diametral strength) by increasing the space available

for ionic bond formation, with ions from the glass particles, and permitting more flexibility in the side chains. Concentration of these polymers was critical with detriment to properties if too high. The same workers also explored the synthesis and incorporation of nano-hydroxyapatite and fluorapatite particles into commercially available glass-ionomer powders. These enhanced mechanical strength. (Xie et al., 2005).

When applied to glass ionomer tethered amino acid residues have the results of

1- Providing greater degrees of freedom for the pendant carboxylate ions to form salt bridges with the Ca^{2+} and Al^{3+} ions released from the glass particles.

2- Enhance the degree of adhesion to tooth structure as a consequence of more availability of $-CO_2H$ residues (Culbertson, 2001).

It is well documented that the powder : liquid ratio at which the cement is mixed impacts on the physical properties (Crisp et al., 1976). Encapsulation reduces the potential for such variation and results in better physical properties (Dowling and Fleming, 2009). In the case of hand-mixed glass ionomers improvements in mixing characteristics result from granulation of glass particles at manufacture (Syrek, 2006). This increases the wetability of particles upon exposure to polyacrylic acid and, from a health and safety perspective, reduces the potential for dust. In addition, such cements are easier to proportion as they adhere less to the manufacturers supplied proportioning spoon (Syrek, 2006).

2.1.4.2 *SOLUBILITY AND EROSION OF GLASS IONOMER CEMENT*

The glass ionomers, zinc polycarboxylate/ phosphate and silicate cements show many similarities in structure as they are formed by acid- base reactions with a highly cross linked ionic matrix. These materials are thought to have equal susceptibility to aqueous or acid erosion. Aqueous erosion values reported using disc specimens made to BSI specification BS3365, are; 0.4 percent for Glass ionomer cement (ASPA IV), 0.5 percent for silicate cement, and 0.2 percent for polycarboxylate cement (Wilson, 1976). Crisp, (1980), found that the glass ionomer cements displayed more resistance to acid erosion *in vitro* than silicate or zinc polycarboxylate cements. Olio (1984) found that the glass ionomer cements were more susceptible to the erosion within the first 5 to 10 minutes of preparation. The rate of erosion of glass ionomer cement declined dramatically thereafter as the time after mixing increased. The amount of erosion of glass ionomer cement is dependent upon the pH of the eroding acidic solution and the stability constants of the complexes formed between aluminium or calcium and the acid anions (Walls, 1986).

2.1.4.3 *FLUORIDE RELEASE*

The fluoride release from some restorative materials has been known to have a localised caries inhibitory effect (Lind et al., 1964). The mechanism of fluoride in preventing caries is complex and not fully understood (Wilson and McLean, 1988). Its anti-caries effect has been attributed to the uptake of fluoride by enamel apatite at hydroxyl sites, and to the high levels of fluoride on the tooth surfaces that increase the resistance to plaque acids. Fluoride may also change the composition of the bacterial plaque and its biochemistry (Wilson and McLean, 1988). The glass

ionomer cements release fluoride by a similar mechanism to that of silicate cements (Mount, 1995). The glass ionomer cements too, including the resin modified types, are capable of releasing considerable amounts of fluoride. Perrin and his co workers (1994) reported that fluoride release from four different types of glass ionomer cements over a year followed a similar trend but differed in amount. In the first few days glass ionomer cements released a large amount of fluoride and thereafter the quantity of released fluoride decreases to a constant amount over a prolonged period of time (Creanor et al., 1994). The rate of fluoride release from glass ionomer cements depends on many factors that are not fully understood (Perrin et al., 1994). Of these the acidity of the surrounding environment and the powder liquid ratio have an effect on the rate of fluoride release (Cranfield et al., 1982). It should also be noted that Creanor and his co workers (1994) reported that the glass ionomer cements also posses an ability to take up fluoride again when its concentration is higher in the environment surrounding the restoration than within it (Creanor et al., 1994).

A mathematical equation has been proposed by Xu and Buryess (2002) to model fluoride releases from both conventional and resin modified glass ionomers.

$$[F]_C = [F]_I (1 - e^{-bt}) + \beta\sqrt{t}$$

Where [F] = Fluoride concentration

C= Cumulative

I= Initial

t= Time. Both b and β are mathematically derived constants.

2.1.4.4 ***ADHESION***

In common with the glass polycarboxylate cements the glass ionomer cements have an ability to adhere permanently to untreated enamel and dentine under moist conditions (Wilson and McLean, 1988). This may be helpful in the restoration of root caries lesions where moisture control is often suboptimal. The exact mechanism of adhesion of glass ionomer cements is unclear (Walls, 1986). Wilson and Kent (1972) thought that the metallic ions in the set cement could make salt bridges between the poly acid and the negatively charged groups on the enamel surface. It seems that the adhesion of the cement with tooth structure occurs following displacement of phosphate ions from the surface of the tooth, with the place of each displaced phosphate ion being replaced with a calcium ion to retain electrolytic balance (Mount, 1998). An elegant mechanism of adhesion to tooth has been described by Van Meerbeek et al. (2006). They describe an initial micromechanical interlocking with the hydroxyl apatite coated collagen fibril network of dentine fostered by the self etch effect of the polyacid component of the glass ionomer. Thereafter substantive ionic bonds are formed between the poly carboxyl groups of the glass ionomers poly acid and the calcium ions of the tooth. In the oral cavity following tooth preparation, the tooth surface is usually covered with a smear layer. An improvement in adhesion between the glass ionomer cements and the cavity enamel and dentine surfaces can be achieved by treating and cleaning the tooth surfaces with agents such as an aqueous solution of tartaric acid, poly (acrylic acid), or dodicin to remove the smear layer and other contaminants that leave the dentinal tubules quite closed but the rest of the surface clean. This also changes the surface energy of the tooth structure to allow better adaptation of the cement and facilitate optimum placement of the restoration (Wilson and McLean, 1988, Mount, 1998). According to Chadwick and

Woolford (1993) the use of tooth cleanser has no statistical effect upon restoration longevity, but is non detrimental to it, thus optimising the chances of restoration survival. Other recent laboratory work supports the use of a poly-alkenoic acid conditioner to enhance bonding of resin modified glass ionomer cements to bur cut dentine as its application removes smear layer interference (Cardosoa *et al.*, 2010).

2.1.4.5 ***BIOCOMPATIBILITY***

In modern medicine and dentistry any material or substance has to be investigated carefully for its biocompatibility prior to clinical application. Many systems are used to evaluate the biocompatibility of materials; *In vivo* systems, where the biological effects of the materials on animals are tested ; *in vitro* systems use cell culture and microorganisms to test biocompatibilities (Leyhausen et al., 1998). For patient and operator safety it is essential to evaluate the biocompatibility of any restorative material, which has a direct contact with living tissues (pulp, dentine, gingival tissue or other oral mucosa) for prolonged periods of time (Sidhu and Schmalz, 2001) .

The conventional glass-ionomer cements posses a number of properties which are claimed to make them biocompatible with oral tissues; (i) low setting exotherm (ii) rapid neutralisation (iii) release of generally benign ions from the set cement (Nicholson et al., 1991). Glass ionomer cements are generally biocompatible with the surrounding tissues with minimal release of organic components (Kuhn et al., 1983, Schmalz et al., 1994). Due, however, to the presence of the monomers hydroxyethyl methacrylate (HEMA) within resin modified glass ionomer they are considered to have less biocompatibility than the conventional cements (Nicholson

and Czarnecka, 2008). In contrast cytotoxicity of freshly mixed glass ionomer cements has been reported by both Dahl and Tronsatad (1976) and Meryon et al (1983), while Kawahara (1979), found that freshly mixed glass ionomer cements inhibited cellular proliferation, but were not cytotoxic. Glass ionomer cements have been shown to bring about mild irritation of the pulp at a level similar to that caused by zinc poly carboxylate and zinc phosphate cements (Plant et al., 1988). Tests upon the pulpal response to glass ionomers in caries-free human premolars demonstrate that the glass ionomer cements cause a greater inflammatory response than zinc-oxide and eugenol cement, but the inflammation resolves spontaneously within 30 days, with no increase in reparative dentine formation (Cooper, 1980, Plant et al., 1984, Tobias et al., 1978).

Cox et al. (1987) tested the effect of a number of restorative materials including glass ionomer cement on a traumatic exposure (non bacterially infected) pulp, and noted a mild inflammatory response of the pulp tissue after a short period, followed by complete healing with dentine bridge formation. The reactions of the exposed pulp to direct contact with freshly mixed glass ionomer cements are severe at the start, but with time pulp inflammation resolves and bacterial layers at the cavity floor are prevented (Sidhu and Schmalz, 2001). With respect to the gingival tissues the response to glass ionomer cements in class V cavities is reported as minimal (Garcia et al., 1981).

Three components of glass ionomer cements can cause adverse effects, the material itself, metals which have been added to the original composition, and non-polymerised resin components (Sidhu and Schmalz, 2001).

The toxicity of components released from the glass ionomer cements has been studied and there is some evidence that the dentine of the tooth prevents diffusion of the strong acids. The sodium, aluminium, silica, phosphate and fluoride are usually released from the conventional glass ionomer under normal conditions but calcium is only released under acidic conditions (Czarnecka et al., 2002). Apart from aluminium these components are considered to be acceptable in the body at the level released from such material. Most attention has been paid to aluminium since it has the ability to be toxic and affect the central nervous system (Yokel and McNamara, 2001). However, the total amount of aluminium released from glass ionomer cements is small and has no adverse biological effect on the body (Czarnecka et al., 2002) . This statement however sits a little at odds with that reported by Reushe et al. (2001). They report upon the use of glass ionomer bone cement used safely many times, in reconstructive otoneurosurgery In one case however death resulted due to aluminium accumulation in the brain arising from failure to close the dura. The resin modified glass ionomer cements are in addition, able to release HEMA (2- hydroxethyl methacrylate). This can penetrate the dentine to the pulp and cause some adverse biological effects ranging from constant inflammation to sensitisation and potential allergic reactions in the patient (Nicholson and Czarnecka, 2008). It is also a known dermatological irritant that is capable of penetrating clinical procedure gloves (Tinsley and Chadwick, 1997) and therefore care must be exercised in the manipulation of the material.

2.1.4.6 *POTENTIAL TO INCORPORATE MEDICAMENTS OTHER THAN FLUORIDE*

Apart from incorporation of fluoride for its therapeutic effect (discussed previously) other agents have been examined.

The conventional glass ionomer cements lend themselves to this as their hydrogel permits release and uptake of such agents and their setting reaction has no appreciable temperature rise to damage incorporated agents (Hatton et al., 2006).

Prentice and Tyas (2006) explored the incorporation of oxalic acid into glass ionomers with the aim of reducing dentine hypersensitivity by its release. It accelerated the set of the material without affecting strength but, due to its low water solubility could only be introduced in low concentration.

Palmer et al. (2004) examined the potential for chlorhexidine release from an experimental glass ionomer cement. This was with the intention of assessing the possibility of its incorporation and release due to its bactericidal effect. Their additions ranged from 0.5 to 13.0% by weight and in proportion to quantity added the working and setting times increased as the compressive strength decreased.

The incorporation of casein phosphopeptide-amorphous calcium phosphate (CPP-AcP) into a self cure glass ionomer was investigated by Al Zraikat et al (2011). This agent inhibits demineralisation and favours remineralisation of tooth substance.

They found that the incorporation of 3% casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) had the potential to improve the cements anticariogenic properties without affecting adversely its mechanical properties.

With regard to promoting cellular adhesion and enhancing biocompatibility, factors relevant to the work presented in this thesis, Choi et al (2008) demonstrated that the addition of a bioactive Sol-Gel glass ($70SiO_2 \cdot 25CaO \cdot 5P_2O_5$) to a commercial glass polyalkenoate (GC,Fuji I cement) produced higher cell viability with no detrimental effect upon the only physical property measured in this work , that of diametral tensile strength. More recent work indicated that the inclusion of bioactive glass improves the biocompatablilty of glass ionomer to fibroblasts (Subbarao et al., 2012).

In this vein Chang et al (2009) explored the effects of adding Type I collagen to promote the cellular adhesion, to glass polyalkenoate, of gingival fibroblasts. Although etching the surface of the material enhanced adhesion the addition of Type I collagen to the cement significantly improved adhesion to these cells. This was not detrimental to the materials compressive strength; indeed it improved it, providing the addition did not exceed 0.01% collagen.

2.1.5 CLINICAL APPLICATION IN DENTISTRY

It is over 30 years since the glass ionomer cements were first introduced into clinical dentistry. The original glass ionomer cements did not receive widespread acceptance until the mid 1980s, especially in the United States (Nicholson, 1997). The clinical development and use of the glass ionomer cement cements was first explained by Mclean and Wilson (1977a, 1977b, 1977c) who were instrumental in the material development. However these materials had some advantages such as ion exchange adhesion to the enamel and dentine, ability to release and up take fluoride ions, and good thermal expansion and contraction (Nicholson, 1997). Original glass ionomer cements had low wear resistance, fractured easily, and required some protection to avoid over-hydration, it is likely that such problems accounted for the slow widespread acceptance of those materials.

Glass ionomer cements now include a wide family of materials that includes direct restoratives, luting agents, liner and bases, as well as pit and fissure sealants. All such products are available in both conventional and resin modified form (Hewlett and Mount, 2003). The differences consist of powder particles size, reactivity of the surface of powder particles, speed of setting and resistance to water loss and uptake. The glass ionomer cements may be classified according to their use in restorative dentistry as; 1-Type I for luting cement. 2-Type II for restoration, 3-Type III lining cement (Mount, 1998).

2.1.5.1 *LUTING CEMENT*

Luting cements have fine powder particles size and are mixed at a low powder-liquid ratio of around 1.5:1 (Mount, 1998). The luting glass ionomer cements are used for cementing stainless steel crowns for deciduous teeth, precision cast crowns fixed prostheses for permanent teeth, space maintainers and single orthodontic bands (Nicholson, 1997). The luting cement used to treat caries-prone patients is glass ionomer because of its ability to release fluoride ions as it is claimed to prevent the development of caries at the restorations margins (Wilson and McLean, 1988).

2.1.5.2 *RESTORATIVE CEMENTS*

There are three different formulations of glass ionomer restorative materials available commercially; auto-cured glass ionomer cements, resin modified glass ionomer cements and reinforced glass ionomer cements (Mount, 1998). The auto cured glass ionomer cements are ideal to restore areas which are not subjected to excessive occlusal load such as the Class V and Class III lesion. The resin modified glass ionomer cements are stronger than the auto-cured glass ionomer and can therefore be used in places where there is a moderate occlusal load. The reinforced glass ionomer cements display higher physical properties but lack translucency. As a consequence their clinical applications are limited to use in places where strength is required more than aesthetics. They may also be used as long-term temporary restorations where their fluoride release is used to stabilise and remineralise cariously affected dentine (Mount, 1998).

In general the restorative glass ionomers are used to restore non carious tooth lesions (erosion/abrasion) without cavity preparation, to restore primary teeth

and Class V and Class III cavities. In addition they may be used as fissure sealants, repair defective margins of restorations, core buildups, and sealing of root surfaces for overdentures (Wilson and McLean, 1988).

Recent guidelines for treatment of dental caries in children (Scottish Dental Clincal Effectiveness Programme SDcepScottish Dental Clincal Effectiveness Programme SDcep, 2010) does not recommend the use of Glass ionomer restorative materials citing the work of Chadwick and Evans (2007)to support this.

In relation to the treatment of root surface caries, the subject of this thesis, the American Academy of Operative Dentistry (The Academy of Operative Dentistry, 2004) recommends glass ionomer as the material of choice when prevention has failed.

2.1.5.3 *LINING AND BASE CEMENTS*

The only difference between the lining and base cement is the powder liquid ratio at which they are mixed. The lining cement is a thin layer of quite low strength cement placed underneath the metallic restoration to work as a thermal insulator and to protect the underling dentine and pulp from traumatisation due to temperature (Mount, 1998). They are also used to seal and obturate occlusal fissures that show early signs of decay (Wilson and McLean, 1988).

2.1.5.4 *CONCLUSION*

The foregoing has reviewed the development, composition and properties of glass ionomer cement. they have widespread clinical application and would appear suitable for modification for root caries treatment.

2.2 ROOT SURFACE CARIES

Root surface caries is defined as " a soft irregular shaped lesion either (1) totally confined to the root surface or (2) involving the undermining of enamel at the cemento-enamel junction but clinically indicating that the lesion initiated on the root surface" (Katz, 1995). Root surface caries usually occurs supragingivally at or close to (within 2mm) of the cemento-enamel junction (CEJ) (Banting, 2001). Experts generally agree that root caries can occur anywhere on the root surface occlusal to the gingival margins but there are contradictory views about root lesions involving the cemento-enamel junction (CEJ). These relate to the classification of such lesions and some think they should be classed as root surface caries extending onto the crown or indeed as coronal caries extending onto the root or even both. This however is more a measurement issue than a diagnostic issue (Banting, 1993).

The occurrence and location of root surface caries is usually associated with age and gingival recession. This is consistent with the idea that root caries occurs in a location close to the crest of the gingiva, where dental plaque accumulates. Root surface caries most commonly occurs on the proximal surfaces followed by the facial surfaces of the tooth (Banting, 2001). In a similar way to coronal caries root surface caries can be classified as an active or inactive (arrested) lesion according to the following criteria:

- **Active root surface caries** appears as well-defined, softened area on the root surface yellow brown in colour. The lesion is usually covered by observable dental plaque, as the root surface caries progresses the surface of this lesion becomes brown or black and is of a "leathery" consistency (Nyvad and Fejerskov, 1986).

- **Inactive (arrested) root surface caries** appears as a dark brown or black coloured lesion with relatively smooth, shiny surfaces which are hard to probing upon application of moderate pressure (Nyvad and Fejerskov, 1986).

2.2.1 PREVALENCE AND INCIDENCE

The prevalence of root surface caries in the general population increases with peoples age (Beck, 1993). This increase in the prevalence of root surface caries is related to the retention of teeth in older people for longer than in previous generations. Also, root surfaces at this age become exposed due to gingival recession putting the root surface at greater risk (Griffin et al., 2004). The occurrences of root surface caries can be prevented using a variety of preventive methods e.g. water fluoridation, and use of fluoridated dentifrices. Maximum efficiency of prevention could be achieved if high-risk individuals were identified earlier and appropriate preventive methods instituted (Ritter et al., 2010).

The evaluation of the prevalence of root surface caries in epidemiological studies of the older population is affected by the presence of fillings on the roots of teeth. Such restorations may have been placed either because of the presence of a carious lesion or due to a cervical non carious wear lesion. However, when conducting the epidemiological studies it is very difficult to discover the reason why a specific surface has been restored (Walls et al., 2000).

Various epidemiological studies have been carried out on different populations, but due to the lack of standardised diagnostic criteria, reporting methods and diversity of the population, inter study comparison is problematical. Some incidence studies have found that around 30-40% of people surveyed have root

surface caries. Many risk factors have been linked to the occurrence of root surface caries and these comprise oral, medical, mental, behavioural and psychological conditions (Galan and Lynch, 1993). Table 2- summaries some of risk factors associated with root surface caries (McCombes, 1999).

Table 2-4 some of risk factors associated with root surface caries.

Risk factors
Oral hygiene state
Frequency of sugar intake
Reduce salivary flow
Total amount of sugars consumed
Mental disability/senility
Physical disability
Poor general health
Cigarette smoking
Presence of a partial denture
Consumption of fizzy drink
Active periodontal disease
Gingival recession
Poor crown margins
Overhanging restoration
Degree of crowding
Presence of erosion
Consumption of alcohol
Number of teeth present
Presence of abrasion cavity

In a National survey of adult health in the Republic of Ireland between 1989 and 1990 a total of 1,527 adult people over 25 were examined for root surface caries It was found that the old people, males, residents of non-fluoridated communities and people of low incomes had the highest prevalence of root surface caries (Whelton et al., 1993)

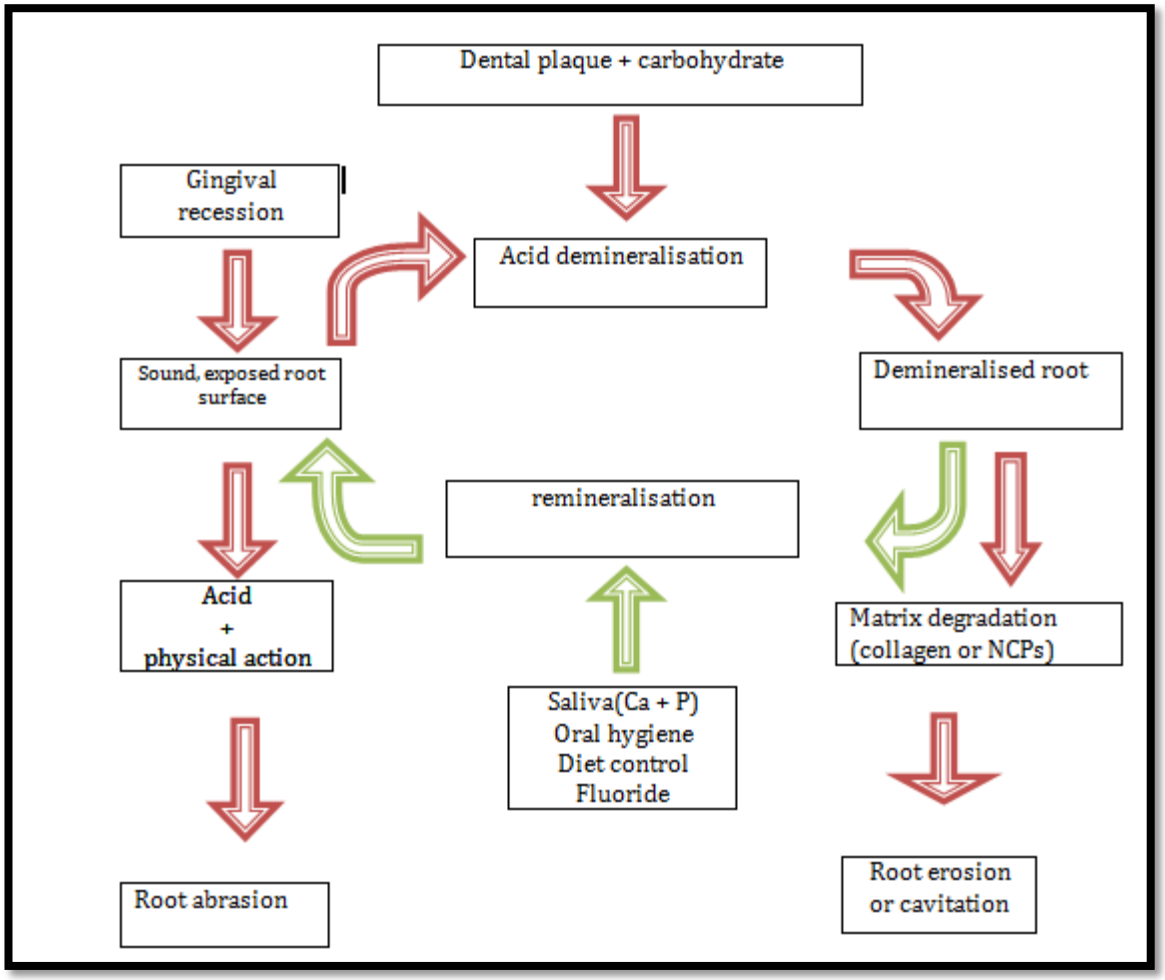
2.2.2 THE PROCESS OF ROOT SURFACE CARIES

Gingival recession, resulting from bad oral hygiene and loss of periodontal ligament attachments with age, leads to exposure of the cement-enamel junction. If

this area retains dental plaque, caries formation is likely (Zhang et al., 2009). The caries process associated with root surface caries is similar to that of coronal caries. Bacteria, able to metabolize carbohydrates into acids, upon exposure to this substrate produce a drop in pH that initiates the decalcification of the tooth structure that they are in contact with (Nyvad and Fejerskov, 1982).

At first there is loss of calcium ions from the crystal apatite of the calcified dental tissues (enamel, dentine and cementum) due to this microbial activity. Under normal conditions, this loss of calcium ions (demineralization) is compensated by the uptake of the calcium ions (remineralisation) by dental calcified tissues from the surrounding oral environment. In an unfavourable environment however, the rate of remineralisation becomes less than the rate of demineralization and the caries process progresses (Banting, 2001).

In root surface caries the demineralization and remineralisation processes involve cementum first, although in some cases the process can start in dentine. There is simultaneous loss of both mineral and protein (proteolysis) from the root tissues so affected (Banting, 2001). Figure 2-5 summaries this process.



2-5 The process of root surface caries (modified from Dung and Liu, (1999)).

Root surface caries is a dynamic process with lesions of different clinical severity and treatment requirements displayed in the same person. The dynamic nature of root surface caries makes the diagnosis of active root caries surface difficult (Lynch and Beighton, 1994).

2.2.3 CLINICAL SIGNS AND SYMPTOMS

The presentation of root surface caries involves different clinical signs that range from slightly softened and discoloured areas to large, yellow-brown soft or hard

areas, which may eventually involve the whole surface. Cavitation may also be present but the pulp may not be involved in an extending cavitated lesion (Fejerskov and Kidd, 2008). The most frequently used clinical signs to describe root surface caries use visual (contour, surface cavitation, colour) and tactile (surface texture) descriptive criteria (Banting, 1993).

Many extrinsic factors affect the clinical appearance of root surface caries. These include the uptake of stain from some dietary foodstuffs, smoking and the micro-flora of the lesion itself (Lynch and Beighton, 1994).

Root surface caries is usually asymptomatic although pain may present where the lesions are advanced (Banting, 1993).

2.2.4 HISTOPAHTOLOGICAL FEATURES OF ROOT SURFACE CARIES

Histological studies of primary root caries reviewed by Lynch and Beighton (1994) clearly show that soft, leathery and hard lesions exhibit different manifestations which reflect the level of bacterial infection of root dentine. The soft lesions are characterised by the presence of bacteria within the dentinal tubules and these spread laterally between the tubules as result of loss of both mineral and organic matrix. Hard lesions are characterised as being fully mineralized with the presence of bacterial ghosts within the amorphous mineral of the remineralised lesion. Leathery lesions show a different appearance than those observed in soft and hard lesions and often there is a fully mineralised surface layer.

Micro radiographically, loss of minerals occurs deep to a fairly well mineralised surface zone, which usually shows mineral content that is higher than that of the unaffected dentine. This high mineral content of the surface zone may reflect a

selective redeposition of minerals in this area, as it has been shown that the size of the apatite crystal in the surface zone is considerably larger than normal cementum (Fejerskov and Kidd, 2008).

At the early stage the root surface caries lesion may appear softened. This is due to the penetration of microorganisms into the surface zone of the lesion between partially demineralised collagen fibres.

At more advanced stages demineralisation usually extends several hundred micrometers into the underlying dentine below the surface. In the case of shallow cavities which may be associated with the root surface caries lesion, the exposed dentine surface may show a relatively well mineralised surface layer below which demineralisation takes place.

2.2.5 DIAGNOSIS OF ROOT SURFACE CARIES

Clinical diagnosis is the process of recognising diseases by their characteristic signs and symptoms (Banting, 2001). The visual (colour, contour, surface cavitation) and tactile (surface texture) signs are the most commonly used signs to recognise root surface caries (Banting, 1993).

Although no connection between the colour and the activity of the root surface caries has been found, it is generally agreed that the discoloration of the root surface indicates the presence of caries (Lynch and Beighton, 1994)

Fejerskov et al. (1991) introduced a classification to assist in the diagnosis of the root surface caries lesion. This combines activity assessment as well as an

evaluation of surface integrity. On the basis root surface caries may be classified as follows:

- Inactive lesion without surface destruction
- Inactive lesion with cavity formation
- Active lesion without definitive surface destruction
- Active lesion with surface destruction (cavitation), but cavity is estimated not to exceed 1 mm in depth (visually)
- Active lesion with a cavity depth exceeding 1 mm , but not involving the pulp
- Lesion expected to penetrate into the pulp
- Filling confined to the root surface or extending from a coronal surface onto the root surface
- Filling with an active (secondary) lesion along the margin
- Filling with an inactive lesion (secondary) confined to the margin

Although the root caries lesion is usually detected by changes in colour (yellow, brown, black), texture (soft, hard), or surface contour (regular, irregular) of the root surfaces, the examination should also focus on the patient's risk of developing root caries. Therefore the diagnosis of the root caries should start by the identification of contributory factors and oral hygiene practices (Berry et al., 2004).

The diagnosis of root surface caries can be carried out visually by gentle gingival tissue displacement with an air syringe and retraction with hand instruments that can give a better view to check subgingival and interproximal areas. The visual diagnostic capability of the root surface caries can be improved by using

transillumination and/or lighted mirrors and intra-oral cameras (Berry et al., 2004). Early interproximal root surface caries can also be detected by radiographs. Vertical bitewing radiographs give better evaluation of inter proximal root surface caries in individual with significant periodontal problems (Jones, 1995).

2.2.6 MANAGEMENT OF ROOT SURFACE CARIES

The management of root surface caries should start by preventive and remineralisation therapies that will help inhibit or eliminate the lesion before further damage to dental tissues occurs. Restorative treatment is indicated in case of excessive distraction of the tooth tissues by active root surface caries (Berry et al., 2004).

2.2.6.1 *NON –OPERATIVE TREATMENT OF ROOT SURFACE CARIES LESION*

It is generally accepted that root surface caries can be prevented or arrested by plaque removal, diet modification and topical fluoride application (Emilson et al., 1993).

Active root surface caries can be converted to inactive lesions only by advising patients to improve their oral hygiene by the use of a toothbrush with fluoride toothpaste (Nyvad and Fejerskov, 1986). Many studies have shown the advantage of substituting dietary polyols for sucrose in chewable dietary items. Xylitol, a five-carbon sugar alcohol, is not metabolized by *Streptococcus mutans* and has been shown to have an anticariogenic effect (Nuuja et al., 1993). Removal of dental plaque from root surface caries has been shown to be a promising factor in arresting the active root lesion (Emilson et al., 1993). A 0.12% chlorhexidine rinse (peridex, Periogurd) can also be helpful in the treatment root surface caries. Chlorhexidine is an antimicrobial solution used to treat gingivitis and periodontal diseases and is also very effective in eliminating cariogenic bacteria (Berry et al., 2004). Despite this there has been relatively limited research into the non-invasive (pharmaceutical) management of root caries. Early studies have demonstrated that an application of ozone for a period of either 10 or 20 seconds is able clinically to change leathery root caries lesions to an inactive lesion (Baysan and Lynch, 2006) although to date there is no independent report to confirm this. It is generally agreed however that fluoride ions promote remineralisation of tooth substance and reduce the rate of demineralisation. The use of a dentifrice with high fluoride content may be considered to reverse primary root caries lesions, since more

fluoride is required for the remineralisation of roots than for enamel (Lynch and Baysan, 2001).

2.2.6.2 *RESTORATIVE TREATMENT OF ROOT SURFACE CARIES*

Some cases of root surface caries do not need restorative treatment for if shallow they can be saucerised using hand instruments, finishing burs or polishing discs (Billings et al., 1985, Wallace et al., 1993).

When the active caries root lesion progresses, it causes destruction of the root tissues and so restorative treatment is indicated to remove the caries and replace the destroyed tissues. Many difficulties face the clinicians during this process. These include impaired visibility, limited access, moisture control, pulpal proximity and the nature of the dentinal tissues themselves (Berry et al., 2004).

Many different restorative materials are used to restore the root surface caries. Glass Ionomer (Conventional, Resin modified) glass ionomer cements are the materials of choice for restorative treatment of most root surface caries lesions (Berry et al., 2004). These materials provide good adhesion to the hard tissues of the tooth and have anticariogenic effects by releasing fluoride over time. Resin composite materials including compomers (polyacid modified resins) and flowable composites are indicated to restore root surface lesions when good aesthetics are required. These materials undergo polymerization shrinkage and some release fluoride (Berry et al., 2004) but as these are resin based they are not moisture tolerant. Amalgam is indicated to restore root surface caries in posterior teeth where aesthetics is unimportant and there is a problem with moisture control (Berry et al., 2004) the use of this material is to be phased down (World Health Organisation, 2010).

Placement of a restoration for a root caries lesion involves the disruption of many tissues (Figure 2.6) namely attached gingiva, periodontal ligament, enamel, dentine and cementum. Scope therefore exists for surface interaction of the restorative material with the cell population of both the attached gingiva and periodontal ligament. This offers the potential for cellular attachment to these tissues to augment the adhesive bonding of glass polyalkenoate to hard tissues of the tooth

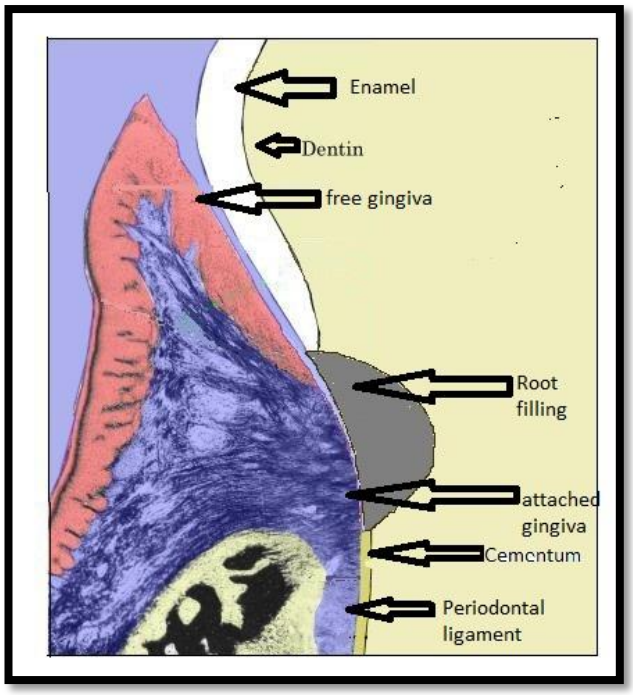


Figure 2-6 The restoration of a root caries lesion showing potential tissue interaction.

The next section of this literature review examines the nature of the attached gingiva for this is the region disrupted by operative root caries treatment and one that could offer cellular interaction with the restorative material.

2.3 GINGIVA

Gingiva is that part of the oral mucosa that surrounds and is attached to the teeth of the maxilla and mandibular covering the alveolar process (Bath-Balogh and Fehrenbach, 2011).

2.3.1 ANATOMY OF THE GINGIVA

The gingival tissue is divided to three anatomical zones; (1) the attached gingiva, (2) inter-dental papillae and (3) free or marginal gingiva (Figure 2-6).

2.3.1.1 *ATTACHED GINGIVA*

Attached gingiva is the portion of the gingival tissues that is tightly attached to the underlying bone and around the neck of the tooth by means of junctional epithelium. The attached gingiva is a masticatory mucosa; pink in colour though it may have some areas of melanin pigmentation. The width of the attached gingiva varies depending on its location. The attached gingiva is separated from alveolar mucosa by the mucogingival junction, which in healthy normal gingiva is located at 3 to 5 mm below the level of the crests (Bath-Balogh and Fehrenbach, 2011).

2.3.1.2 *INTER-DENTAL PAPILLAE*

Inter-dental papillae are an extension of the attached gingiva located between adjacent teeth and fill in the space between the teeth apical to their contact. The inter dental-papillae are a conical shape around anterior teeth but have a blunt

shape buccolingually in the case of posterior teeth (Bath-Balogh and Fehrenbach, 2011).

2.3.1.3 *FREE OR MARGINAL GINGIVA*

Free or marginal gingiva is the narrow rim of the mucosa that is not attached to the underlying bone and is located just coronal to the attached gingiva. The free or marginal gingiva is bound on its margin by the gingival sulcus, which separates it from the tooth, on its outer margin by the oral cavity and apically at its free surface by the free gingiva. The free gingiva is separated from the attached gingiva by the free gingival groove; the depth of this groove varies according to anatomical site. The groove is very clearly pronounced on mandibular anterior and premolars (Bath-Balogh and Fehrenbach, 2011, Avery and James, 2000).

2.3.2 HISTOLOGY OF GINGIVAL TISSUE

Although the attached and marginal gingiva has some similar histology each one has specific histological features.

The attached gingiva is formed from a thick layer of mainly parakeratinised stratified squamous epithelium, which obscures its extensive blood supply in the lamina propria, making the attached gingiva tissue look pinkish in colour. The lamina propria also consists of tall, narrow connective tissues, papilla and rete ridges that manifest as different amounts of stippling in the overlying gingiva. Therefore, the interface between the epithelium and lamina propria is highly interdigitated. The lamina propria is directly connected to the underlying bone,

making the attached gingiva firm and fixed, and thus serves as a mucoperiosteum (Bath-Balogh and Fehrenbach, 2011).

The marginal gingiva is a masticatory mucosa consisting of a surface layer of orthokeratinised stratified squamous epithelium. The connected lamina propria also has tall, narrow papillae. However this lamina propria is continuous with the lamina propria of the gingival tissue that opposes the tooth surface. The marginal gingiva is not connected to the underlying bone, making this part of the gingival tissue firm but mobile. The gingival fibre group is located in the lamina propria of the marginal gingiva. The gingival fibre group is considered as a part of the periodontal ligament by some histologists, but the gingival fibre group supports only the gingival tissue and not the tooth in relationship to the alveolar bone. The lamina propria of the marginal gingiva is also continuous with the adjacent connective tissue, which consists of the lamina propria of the attached gingiva and the periodontal ligaments (Bath-Balogh and Fehrenbach, 2011).

2.3.2.1 ***DENTOGINGIVAL JUNCTION TISSUE***

The connection between the tooth surface and the gingival tissues is called the dentogingival junction. During the clinical examination of the healthy gingival tissue it is difficult to distinguish between the sulcular epithelium and junctional epithelium.

The cervicular epithelium or sulcular epithelium is located away from the tooth surface, forming a gingival sulcus, which is filled with gingival cervicular fluid (GCF). The depth of the healthy gingival sulcus range between 0.5 to 3mm, with average of 1.8mm (Bath-Balogh and Fehrenbach, 2011).

The junctional epithelium (JE) is the deeper extension of the sulcular epithelium, which lines the floor of the gingival sulcus and is attached to the tooth surface by means of an epithelial attachment. The junctional epithelium can be attached to the enamel, cementum, or dentine. The location of the epithelial attachment (EA) on the tooth surface is initially on the cervical portion of the anatomical crown when the tooth becomes functional after eruption (Bath-Balogh and Fehrenbach, 2011).

2.3.2.2 *HISTOLOGY OF DENTOGINGIVAL JUNCTIONAL TISSUE*

Similar to the epithelium of the attached gingiva and adjacent outer marginal gingiva, the sulcular epithelium consists of stratified squamous epithelium. However, the sulcular epithelium is either nonkeratinised stratified squamous or para-keratinised stratified squamous epithelium with its cells tightly packed, unlike the keratinized marginal gingiva and attached gingiva (Bath-Balogh and Fehrenbach, 2011).

The interface between the sulcular epithelium and the lamina propria that it shares with the outer gingival are fairly smooth, compared with others the deeper interface between the junctional epithelium and the underlying lamina propria is also relatively smooth, without are ridges or connective tissue papillae. The junctional epithelial cells are loosely packed, with less desmosomal junction between cells comparing with other gingival tissues, therefore the number of intercellular spaces between the epithelial cells of the junctional epithelium is increased. The intercellular spaces allow mobile white blood cells (WBCs) to move from the blood vessels in the lamina propria into junctional epithelium.

The junctional epithelium is thinner than the sulcular epithelium, ranging coronally from 15 to 30 cells at the floor of the gingival sulcus, tapering apically to 3 to 4 cells at the apical portion. The superficial or suprabasal epithelial cells of the junctional epithelium serve as part of the epithelial attachment of the gingiva to the tooth surface**.** The suprabasal epithelial cells of the junctional epithelium provide the hemidesmosomes and an internal basal lamina that gives the epithelial attachment since this is a cell-to-noncellular type of cellular junction. The structure of the epithelial attachment is similar to that of the junction between the epithelium and subadjacent connective tissue. The internal basal lamina consists of a lamina lucida and lamina densa (Bath-Balogh and Fehrenbach, 2011).

This internal basal lamina of the epithelial attachment is continuous with the external basal lamina between the junctional epithelium and the lamina propria at the apical extent of junctional epithelium. The epithelial attachment is very strong in a healthy state, acting as a type of seal between the soft gingival tissues and the hard tooth surface(Bath-Balogh and Fehrenbach, 2011).

The deepest layer of the junctional epithelium or basal layer undergoes constant and rapid cell division, or mitosis. This allows a constant coronal migration as the cells die and are shed into the sulcus. The few layers present in the junctional epithelium, from its basal layer to the suprabasal or superficial layer, do not show changes in cellular appearance related to maturation, unlike other gingival tissues. They do not mature into a granular layer or intermediate layer. Thus, junctional epithelium does not mature like keratinized tissue, which fills it is maturate superfacial cells with keratin. Nor does junctional epithelium mature like nonkeratinised tissue, which enlarges its cells as they mature and migrate superficially (Bath-Balogh and Fehrenbach, 2011).

2.4 PERIODONTAL LIGAMENT (PDL)

The periodontal ligament (PDL) is that part of periodontium that provides for attachment of teeth to surrounding alveolar bone using the cementum (Bath-Balogh and Fehrenbach, 2011). It is a specialized soft connective tissue situated between the tooth root and alveolar bone. Its thickness ranges between 0.15 to 0.38 mm. The thinnest part of PDL is located around the middle part of the root. With age the thickness of PDL progressively becomes thinner with age. The principle function of the PDL is to connect the tooth to the alveolar bone socket and simultaneously provide a cushion enabling them to cope with the masticatory forces applied on them. However, the PDL also acts as a reservoir of cells necessary for tissue healing and regeneration (Nanci and Bosshardt, 2006).

2.4.1 DEVELOPMENT OF THE PERIODONTAL LIGAMENT

PDL development starts when root formation begins as it is shown in Figure (2-7). This is prior to tooth eruption. Hertwig's epithelial root sheath (HERS) grows in an apical direction separating cells of the dental papilla from those of the dental follicle located between the alveolar bone and the outer side of HERS. This sheath forms as a result of the continuous growth of the inner and external enamel epithelia initially forming the cervical loop of the tooth bud that, later on, matures into HERS (Cho and Garant, 2000).

Two subpopulations of mesenchymal cells make up the cellular component of the dental follicle. These are the mesenchymal cells of dental follicle proper and the perifollicular mesenchyme. Each subpopulation is morphologically and spatially distinct from the other. The perifollicular mesenchyme is located between the

dental follicle proper and the alveolar bone. Its cells are small, unorganized, scattered and star-shaped (Cho and Garant, 2000). Their small cytoplasm contains inactive Golgi complex, a small number of mitochondria, rough endoplasmic reticulum and free ribosomes. These cells connect with each other through long and fine cytoplasmic processes and are surrounded by a small number of short collagen fibrils. In parallel with root formation, cells of the perifollicular mesenchyme begin the process of synthesizing and depositing the extracellular matrix collagen fibrils and glycoproteins of the nascent periodontal ligament as a result of an increase in size, polarity and synthetic activity of these cells. These cells gain an elongated morphology, higher numbers of rough endoplasmic reticulum and mitochondria and an active Golgi complex (Cho and Garant, 1989 , Freeman and ten Cate, 1971). The PDL space between the alveolar bone and cementum that was initially occupied by an unorganized connective tissue becomes remodelled, and the provisional extra cellular matrix (ECM) matures into a system of organized fibre bundles that extend between the alveolar bone and cementum surfaces. This remodelled PDL can now provide a firm attachment of the tooth to bone. Later on, eruptive and occlusal forces will further reorganize the PDL (Nanci and Bosshardt, 2006).

Undifferentiated stem cells are present in both developing and mature PDL tissues. They have to the potential to differentiate into fibroblasts, cementoblasts, or osteoblasts (Wallace et al., 1993, McCulloch, 1985). However, whether or not these cells differentiate from a common progenitor or from a specific line of undifferentiated stem cells is still unknown.

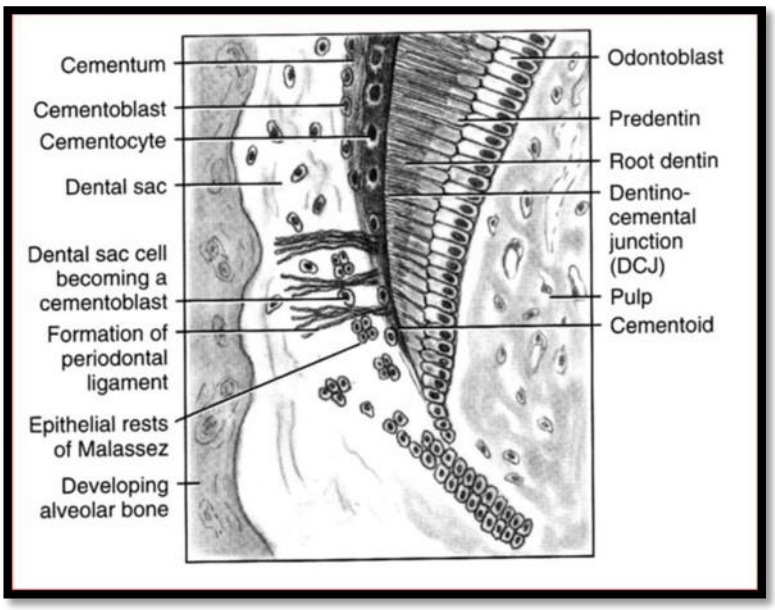


Figure 2-7 PDL development and root formation modified from (2014)

2.4.2 CELLULAR COMPONENTS OF THE PERIODONTAL LIGAMENT

2.4.2.1 *FIBROBLASTS*

The fibroblast cells are the most common cells in all cells of the PDL. Despite the microscopic morphological similarity amongst them, fibroblasts of different connective tissues as well as those of the same connective tissue are composed of a heterogeneous mix of cell populations. PDL fibroblasts have a rapid turnover of the ECM, especially collagen. PDL fibroblasts are active cells that synthesize and deposit proteins of the ECM. They have a large size with a cytoplasm rich in rough endoplasmic reticulum, Golgi complex, and many secretary vesicles (Nanci, 2013). The PDL is subjected to mechanical forces and strains. These strains determine the morphology and synthetic activity of PDL fibroblasts. They also determine the interaction of PDL fibroblasts with its surrounding ECM including adhesion. PDL fibroblasts show a prolonged, polarized cytoplasm extensively contacting with collagen fibrils of the surrounding ECM (Garant and Cho, 1979, Beertsen et al., 1979). The presence of a highly organized cytoskeleton, which has a well-developed network of actin, provides these cells with the capability to migrate and change their shape in coping with the functional demands placed on them. PDL fibroblasts frequently contact with each other through gap and adherens junctions. Their alignment follows the orientation of the fibre bundles, and they attach themselves to these fibres by wrapping their cytoplasmic processes around them. PDL fibroblasts have the ability to produce and degrade collagen. This ability allows them to remodel bundles of collagen fibrils in a continuous manner. Since the collagen turnover is unusually rapid, any disease process that causes an

impaired PDL fibroblast function results in an accelerated loss of the tooth-supporting tissues. Periodontal lesions of an inflammatory nature such as periodontitis are associated with an increased expression of matrix metalloproteinases (MMPs) that causes a massive destruction of collagen. Therefore, therapeutic strategies that involve MMP inhibition may be advantageous in controlling periodontal disease (Nanci, 2013).

2.4.2.2 *EPITHELIAL CELLS*

The PDL epithelial cells are known as the epithelial rests of Malassez. They are remnants of the Hertwig's epithelial root sheath. They are seen as clusters of epithelial cells with deeply stained nuclei in hematoxylin-and-eosin-stained sections near the cementum especially in furcation areas of multi-rooted teeth (Nanci, 2013, Nanci and Bosshardt, 2006). The function of these cells is unknown but they have been speculated to play a role in periodontal regeneration and repair (Bosshardt, 2005).

2.4.2.3 *UNDIFFERENTIATED MESENCHYMAL CELLS*

Human PDL has been shown to contain cell subpopulation with stem-cell characteristics (Delima et al., 2002). They are referred to as undifferentiated mesenchymal stem cells (UMSCs), or progenitor cells. The production of new PDL cells is thought to be balanced by selective apoptosis of older PDL cells, as PDL cell number is known to be in a state of steadiness. PDL provides cells for its own healing as well as cells that help in the regeneration of adjacent alveolar bone and root cementum (Freeman and ten Cate, 1971, Gillard et al., 1977).

2.4.2.4 *OTHER CELLS OF THE PERIODONTAL LIGAMENT*

In addition to the main cells of PDL (PDL fibroblasts, epithelial rests of Malassez, and UMSCs), PDL also contains other cell types such as osteoblasts and osteoclasts (physically present in the ligament but functionally associated with alveolar bone) monocytes, macrophages, odontoclasts, and cementoblasts (although present in the ligament, they are functionally associated with cementum) (Nanci, 2013).

2.4.3 EXTRACELLULAR MATRIX

The extracellular matrix is a complex of different proteins and glycosaminoglycan families defining structural integrity and a variety of physiological function. The main function of extracellular matrix is to provide tissues with their specific biochemical and biomechanical properties. The local cells are responsible for synthesis secretion and maintenance of extracellular matrix (Gelse et al., 2003). Fibroblasts synthesise and secrets different extracellular molecules. These consist of the components of the fibres, the component of the ground substance (extrafibrillar matrix) and a variety of biologically active molecules, such as cytokines and growth factors (Nanci, 2013).

2.4.3.1 *COLLAGEN FIBRES*

Collagen is one of the most abundant proteins in the body (Nanci, 2013). Made from very long fibrils with a characteristic axial periodic structure. The fibrils offer the major biomechanical scaffold for cell attachment and anchorage of

macromolecules, allowing the shape and form of tissues to be defined and maintained (Kadler et al., 1996). The name collagen is a general term that refers to proteins forming a characteristic triple helix of three polypeptide chains and all members of the collagen family form these supramolecular structures in the extracellular matrix (Gelse et al., 2003). The name collagen derives from the greek meaning literally animal glue.

Collagen is different from other proteins in that the molecule contains three polypeptide chains (α-chains) which form a unique triple-helical structure. For the three chains to wind into a triple helix they must have the smallest amino acid, glycine, at every third residue along each chain. Each of the three chains therefore has the repeating structure Gly-X-Y, in which X and Y mean any of the 20 amino acids in proteins but are usualy the imino acids proline and hydroxyproline (Kadler et al., 1996). At least 29 types of collagen have been identified (Bath-Balogh and Fehrenbach, 2011).

The collagens can be subdivided according to their structure and supramolecular organization into :- (Nanci, 2013)

- Fibrillar collagens the include types I, II, III, V, XI, XXXIV and XXVII. These types of collagens aggregate in a highly organised way to produce fibrils with a typical 62-nm banding pattern.
- Basal lamina collagen includes type IV collagen. The size of collagen IV is similar collagen type I, but does not assemble in fibrils.
- Fibril-associated collagen includes types IX, XII, XIV, XIX, XX, XXI and XXII collagens. These types are found in different location in different tissues for example type XIX collagen found in basal laminae and it is important for skeletal muscle cell differentiation.

- Network–forming collagen includes type VIII and X collagens. Type VIII is believed to provide compressive strength while providing an open, porous meshwork. Type is X usually found in the hypertrophic zone of the epiphyseal cartilage growth plate.
- Anchoring-fibril collagen includes type VII collagen which form an anchoring fibrils extend from the basal lamina into the underlying connective tissues.
- Microfibirl-forming collagen includes type VI collagen which is found in most connective tissues
- Transmembrane collagens includes type XIII, XVII, XXIII, and XXV. Type XVII collagen is present in hemidesmosomes of basal epidermal cells and attaches the cells to the basal lamina. Type XIII is found in adhesion site of fibroblast and at cell matrix interfaces in some epithelia, muscles, and nerves.
- Multiplexin (endostatin –forming) collagens includes type XVIII and XV are found in association to basement membranes, they can mediate cell adhesion to different collagen receptors.
- Other collagens include type XXVI and XXVIII. Type XXVI is present in extracellular matrix of testis and ovary. Type XXVIII is found in the basement membrane around Schwan's cells of the peripheral nervous system and dorsal ganglia.

2.4.3.1.1 TYPE I COLLAGEN

Type I collagen is the most predominate collagen in vertebrates forming the major parts of the fibrillar extracellular matrix (Stamov and Pompe, 2012). Thus, it has

become an almost ubiquitous biomolecule to use in modern biomimetic cell culture scaffolds and in tissue engineering scenarios, where new functions for biomedical applications are sought (Stamov and Pompe, 2012). Type I collagen comprises more than 90% of the organic mass of bone and is the main collagen of tendons, skin, and ligaments (Gelse et al., 2003). Fibroblasts, odontoblasts, and osteoblasts synthesise and produce the same type I collagen. As a secretary protein, fibrous collagen is synthesised as a proprotein (*procollagen*) in a similar way to secretary proteins of other cells (Nanci, 2013). Type I collagen triple helix is usually formed as a heterotrimer by two s identical α1 (I) - chains and one α 2(I)-chain (Gelse et al., 2003). The type I collagen fibrils usually have small amount of collagen type III, V, and, XII (Nanci, 2013). The major function of type I collagen is to provide structural integrity and tensile strength to connective tissues (Nanci, 2013).

2.4.3.2 ***GROUND SUBSTANCE (EXTRAFIBRILLAR MATRIX)***

The ground substance of extracellular matrix appears as amorphous gelatinous substance in the microscope. It contains a complex mixture of macromolecules with important function. These macromolecules interact with cells and fibrous components of the matrix and play some roles in adhesion and singling events. The ground substance of the PDL plays an important role in providing the tooth with its capability to endure occlusal forces placed on it. Water has been found to comprise about 70% of the ground substance's composition. During inflammation or trauma, tissue fluids of the ground substance increase within its amorphous matrix (Nanci and Bosshardt, 2006). Fibroblasts synthesis and secret two main types of

macromolecules which form the ground substance: *proteoglycans* and *glycoproteins (Nanci, 2013).*

2.4.3.2.1 PROTEOGLYCANS

They are a large group of extracellular matrix and cell surface-associated molecules that consist of a protein core to which *glycosaminoglycan* chains are connected. Glycosaminoglycans are long chains of repeating disaccharide units consisting of a hexosamine and uronic acid, several different glycosaminoglycans are recognized depending on the combination of hexosamine and uronic acid. The main property of cell surface matrix proteoglycans is their capability to attach growth factors, cytokines, and other biologically active molecules (Nanci, 2013).

2.4.3.2.2 GLYCOPROTEINS

Several glycoproteins can be found in the ground substance such as fibronectin, tenascin and thrombospondine; a number of these have adhesive functions. The main function of glycoproteins is to attach cells to extracellular matrix (Nanci, 2013).

2.4.3.2.2.1 FIBRONECTIN

Fibronectin is a main extracellular matrix and plasma glycoprotein synthesised by hepatocytes and fibroblasts (Nanci, 2013). Fibronectin is a complex glycoprotein that is thought to improve the attachment of connective tissue cells to collagen fibrils as well as other components of the extracellular matrix (Jones et al., 1986). It is believed to facilitate the migratory process of connective tissue cells since

these cells seem to avidly attach to fibronectin. Therefore, considering the rapid turnover of the PDL, one can conclude that PDL fibronectin might possess highly significant biological actions (Berkovitz, 1990). Fibronectin and type I collagen have been found to be produced by 99% of the ligament cells, while the remainder of PDL cells produce fibronectin only (Connor et al., 1983).

Electron microscopic studies have shown fibronectin to be present over collagen fibers and at points of contact between cells and collagen (Pitaru et al., 1987). Fibronectin becomes lost from the ground substance of many connective tissues as they mature (Hassell et al., 1978, Linder et al., 1975). Thus, the persistence of PDL fibronectin within its ground substance may suggest a failure of PDL to attain maturity (Berkovitz, 1990). Fibronectin exists as a protein dimer, composed of two nearly identical polypeptide chains linked covalently by a pair of C- terminal disulfide bonds. Each fibronectin monomer contains three types of repeating units type I, II, and III (Pankov and Yamada, 2002). A large number of different integrins attach to fibronectin including the classic fibronectin receptor a5b1 (middle panel, Integrin interaction sites). Thorough analyses have reduced the regions associated with cell adhesion along the lengthy fibronictin molecule to several minimal integrin-recognition sequences. The best known is RGD sequence (Arg–Gly–Asp) which is located in fibronectin repeat III10 (Pankov and Yamada, 2002).

2.5 ARGININE-GLYCINE-ASPARTIC ACID (RGD)

The arginine-glycine-aspartic acid (RGD) cell adhesion sequence was found in fibronectin in 1984 (Pierschbacher and Ruoslahti, 1984). It is the most effective and widely used peptide to promote cell adhesion on a synthetic surface. This is based upon its widespread distribution and use throughout the organism, its

capability to label more than one cell adhesion receptor, and its biological effect on cell anchoring, behaviour and survival (Hersel et al., 2003).

The RGD is a small structure and can be reproduced with peptides, indeed this is how the site was originally discovered (Ruoslahti, 1996). The RGD peptides stimulate cell adhesion when they are immobilised on surfaces (Hersel et al., 2003), whereas in solution they act as decoys inhibiting adhesion. (Ruoslahti, 1996).

RGD peptides were also found in many other extracellular matrixes such as vitronectin, fibrinogen, von Willebrand factor, collagens, laminin, osteopontin, tenascin and bone sialoprotein as well as in membrane proteins, in viral and bacterial proteins, and in snake venoms (Hersel et al., 2003). The connection of the cells to neighbouring cells and the surrounding extracellular matrix are usually mediated by Cell adhesion receptors. The intergin receptors play a major role in anchoring molecules and also they are important in other process such as embryogenesis, cell differentiation, immune response, wound healing and hemostasis (Ruoslahti and Pierschbacher, 1987). Integrins include two non-covalently connected transmembrane subunits, termed a and b: To date 18 a and 8 b subunits are known, that makes 24 different heterodimers (Van der Flier and Sonnenberg, 2001).

The combination of the particular a and b subunits decide the ligand specificity of the integrin. Some integrins, however, are highly promiscuous, e.g. the avb3 integrin links to vitronectin, fibronectin, von Willebrand factor, osteopontin, tenascin, bone sialopro sialoprotein and thrombospondin. *Vice versa* ECM molecules, like fibronectin, are ligands for several integrins (Van der Flier and Sonnenberg, 2001). In an attempt to decrease macromolecular ligands to small

recognition sequences, the tripeptide motif RGD was found as an important cell adhesion peptide sequence in fibronectin (Hersel et al., 2003).

In several cases RGD peptides were proven to promote cell adhesion on various materials (Hersel et al., 2003). RGD as a mediator to cell adhesion is tested often on new polymers to prove their applicability as bioactive materials. More studies using established polymers revealed the ability of RGD peptides to mediate cell adhesion in vivo, thus leading e.g. to enhanced bone in growth and decreased fibrous encapsulation of bone implants (Eid et al., 2001, Kantlehner et al., 2000). Adhesive peptides have found several applications such as surface-coated RGD peptides are being tested to improve the compatibility of different implant devices (Ruoslahti, 1996).

2.6 ENAMEL MATRIX DERIVATIVE (EMDOGAIN)

Enamel matrix derivative (EMD) is an acid extract of pig enamel matrix (Emdogain_; Straumann AG, Basel, Switzerland) and has been successfully used to stimulate functional periodontal ligament, cementum and alveolar bone to regrow in patients with severe periodontal attachment loss (Lyngstadaas et al., 2009). The enamel matrix protein in combination with an aqueous solution of propylene glycol alginate (PGA) called Emdogain has been on the market in Europe, USA, and Japan for 12 to 13 years, offers good clinical results after periodontal surgery of intraosseous defects (Gestrelius et al., 2000).

2.6.1 COMPOSITION OF ENAMEL MATRIX PROTEIN AND ITS DERIVATIVE

The enamel matrix consists of several proteins such as amelogenin, amelin (also called ameloblastin or sheathlin) enamelin, tuft protein, proteases, and albumen (Gestrelius et al., 2000).

Amelogenins are the main component of enamel matrix protein, which are a family of hydrophobic proteins that account for more than 90% of the organic constituents of the enamel matrix (Romanelli et al., 2008). The amelogenins are known to self-assemble into supramolecular aggregates that form an insoluble extracellular matrix (Fincham et al., 1994) with high affinity for hydroxyapatite and collagens (Gestrelius et al., 1997).

Enamelines are the second most common component of the enamel matrix protein, they have been found to have serum proteins, and the more general term "non-amelogenin" is now commonly used to illustrate this high molecular weight fraction, which comprises proline-rich enamelin, tuftelin, and tuft proteins (Rathva, 2011).

Three matrix proteins (amelogenin, enamelin, sheathelin) and two enzymes (MMP-20, EMSP1) have been extracted and the cDNA replicated from developing porcine teeth (Rathva, 2011). Although early immunoassay studies could not detect the presence of growth factors in EMD (Gestrelius, 1997) nominal levels of transforming growth factor β1 have been identified immunologically (Kawase et al., 2001). In addition by using noggin, a bone morphogenic protein (BMP)-binding protein, investigators have found BMP-2 and BMP-4 in an osteoinductive fraction of enamel extracts (Iwata et al., 2002)

2.6.2 MECHANISM OF ACTION

The effect of enamel proteins on periodontal ligament formation is supported by their presence in early cementum formation during development of tooth attachment (Spahr and Hammarstrom, 1999, Hammarstrom, 1997) However, the mechanism(s) of how EMD stimulates periodontal regeneration is not fully understood (Lyngstadaas et al., 2009). The enamel matrix is thought to have a regulatory effect on the initiation, propagation and maturation of hydroxyapatite crystallites in enamel. Temporary deposition of extracellular matrix onto the dentinal root surface gives an initial and important effect on a cellular cementum formation (Slavkin and Diekwisch, 1997).

The formation of tooth root is started by downgrowth of the Hertwig's epithelial root sheath (HERS). This contains two layers of cells, of which the inner layer is the apical extension of the enamel organ. The enamel protein matrix is synthesized and secreted by ameloblasts. Recently it has been found that the cells of the Hertwig's epithelial root sheath have the ability to secrete and deposit enamel matrix proteins on the root surface before cementum formation (Hammarstrom, 1997, Fong et al., 1996). There is some evidence that the deposition of enamel matrix protein on the apical end of the root works as an initiating a factor for cementum formation (Hammarstrom, 1997). The formation of the cementum is usually associated with the development of the periodontal ligament and the alveolar bone (Gestrelius et al., 2000) .

A number of studies have been conducted to understand the mechanism of action of emdogain. In these immunoassays for different growth factors, cytokines and attachment protein were all negative (Gestrelius et al., 1997). For better

understanding of the effect of the cellular level, enamel matrix protein was added to human periodontal ligament cells culture and monitored for cellular attachment rate, proliferation, DNA replication and metabolism. These studies revealed that the enamel matrix protein enhanced proliferation of periodontal ligaments cells, as well as increased protein and collagen production and mineralization (Gestrelius, 1997) PDL fibroblasts modified with EMD showed an increased intracellular cAMP concentration and autocrine releasing of TGF- _ 1, IL-6 and PDGF AB compared to the control group (Lyngstadaas et al., 2001). However the enamel protein matrix had insignificant effect on epithelial cell proliferation in vitro (Gestrelius, 1997). It was concluded that the application of enamel matrix protein on root surface may inhibit epithelial perforation in a same way of the mechanical prevention achieved using a barrier membrane in guided tissue regeneration procedures (Gottlow et al., 1986, Nyman et al., 1982).

2.6.2.1 **LITERATURE REVIEW CONCLUSION**

The foregoing has reviewed potential bioactive material which could facilitate the production of new dental restoratives that could enhance their capacity to biologically interact with the surrounding tooth and supporting structures to enhance durability and deprive residual caries of its nutrient supply. Aspects of this is explored in the work that follows.

- **AIMS**

1. Ascertain UK and Libyan dentists' management strategies for root caries.

2. Identify agents capable of promoting cellular attachment to glass ionomer cement and determine effective dosage levels.

3. Assess the effects of addition to some physical properties of commercially available glass ionomer.

3 MATERIALS AND METHODS

This work sought to find biologically active agents that had the capacity to promote cellular attachment of gingival tissue cells to glass ionomers with the aim of preventing the ingress of cariogenic nutrients to subgingival glass ionomer restored root caries surface lesions. The work did not ascertain the clinical effectiveness but focused upon identifying such agents and assessing their effects of their addition upon some physical properties, of importance for clinical success, of two commercially available glass ionomers. Two strands of work were undertaken.

1- A survey of UK and Libyan dentists to ascertain their current practice as regards management of root caries
2- A laboratory study that identified agents capable of promoting cellular attachment to glass ionomers, determined effective dosage levels and assessed the effects of addition to some physical properties of commercially available glass ionomer.

3.1 QUESTIONNAIRE METHODOLOGY

A postal questionnaire and covering letters were designed according to the principles of Dillman (1978) for postal distribution to qualified dentists in both the United Kingdom and Libya. Before finalization the questionnaire was piloted upon convenience samples of the target groups to maximize the clarity of the questionnaire. Once in final form a copy was sent to the Scientific Officer of the Research Ethics Service of Fife, Forth Valley and Tayside, with the proposed covering letters, to determine if ethical approval was required. The reply received stated that the work proposed did not require ethical review under the terms of the Governance Arrangement for Research Ethics Committees GAfREC in the UK (Appendix A). No ethical approval is required in Libya.

The questionnaire was constructed (Appendix B) to permit anonymous return and was distributed nationally, within the UK by post to 400 registered dentists. 200 copies of the questionnaire were handed directly to 200 registered dentists in Libya by the author calling personally at the largest multiple practices in Tripoli, Al-zawia and Sabratha in January 2011.

400 Potential participants in the United Kingdom were randomly selected from the electronic registers, held by the General Dental Council on February, 2009. Randomization was achieved by sequentially numbering each entry in the electronic versions of the registers and selecting those whose number coincided with a list of random numbers produced using the random number function in the spreadsheet package Excel (Microsoft Excel 2002, Microsoft Ltd., Reading UK). An introductory letter and stamped addressed return envelope for the completed questionnaires were included in the mailing (Appendix C). The introductory letter invited the potential participants to take part and stated that non return of the

questionnaire would be indicative of a lack of consent to participate in the study. To ensure maximum confidentiality of all respondents no hidden codes were embedded in any part of the questionnaires and, so it was impossible to know who had participated. For this reason it was impossible to send follow up letters to non responders.

The questionnaires sought the views and experiences of root surface caries management of dentists in both United Kingdom and Libya.

A relational database was constructed using the computer programme Paradox (Paradox Version 3.5, Borland International, CA 95067 - 001, USA) for input of data from the completed questionnaires and interrogation. Statistical analyses of the responses were undertaken using Prism (Graph Pad Prism, Version 4, Graph Pad Software Inc., San Diego, CA92121, USA) and Excel (Microsoft Excel, 2002, Microsoft Ltd., Reading UK).

In some cases the questionnaire allowed the respondent to select an unknown response by ticking “other” than describing what this referred to. This was included to maximize the chances of obtaining all information. To facilitate analysis all such responses were filtered as follows;

1. If the response description for “other” was one or more of the categories on the questionnaire such a response was transferred to those categories

2. If the response of “other” had no description it was classified as “other” response.

3. If the response of “other” had a description it was classified according to the description and not included in “other”.

3.2 LABORATORY INVESTIGATIONS

The laboratory component of this work sought to identify bioactive additions, potentially suitable for chairside incorporation at the time of mixing into glass poyalkenoate cement, to foster cellular attachment to subgingival restorations of root caries lesions. Although not tested in this thesis the purpose of these additions was to provide a tissue seal for such restorations with a view to depriving residual cariogenic bacteria of their nutrient supply. In addition, such additions could potentially affect the physical properties of the materials so these were determined for each material with no additions and also, in the case of bioactive additions shown to promote cellular attachment.

3.2.1 INVESTIGATION OF BIOACTIVE ADDITIONS TO GLASS POLYALKENOATE CEMENTS

Prior to assessing the effects of biological additions upon the properties of glass ionomer cements their biological properties were determined using a variety of methods.

Observation under the microscope

- Determination of the optimal materials for attachment of cells.
- Determination of the optimal bioactive additives and concentration for attachment of cells

Cell viability testing (MTT Assay)

- Determination of optimal material for attachment of cells
- Determination of optimal bioactive additives and concentration

Immunocytochemistry (ICC)

Protein biochemistry

- Cell lysis
- SDS PAGE (Polyacrylamide gel electrophorsis)
- Western blotting

3.2.1.1 *OBSERVATION UNDER THE MICROSCOPE*

In order to evaluate the visible effect of materials and bio-modified materials on oral mucosa fibroblast cells, the cells were cultured along with specimens for 21 days during this period the cells were observed and monitored under a light microscope and pictures were taken every 3 days.

3.2.1.1.1 DETERMINATION OF THE OPTIMAL MATERIALS FOR ATTACHMENT OF CELLS.

- **MATERIALS AND METHODS**

Two market leading conventional glass polyalkenoate cements, suitable for restoration of root caries lesions, were selected. Details of these materials are contained in Table 3.1.

Table 3-1 The details of the materials used in the study.

	ChemFil Superior		**GC Fuji VIII GP**	
Manufacturer	DENTSPLY DETREY GmbH 78467 Konstanz GERMANY		GC CORPORTAION 76-1 HASUNUMA-CHO, ITABSHI, TOKYO, JAPAN	
Composition	**Powder (1g)**	-Aluminium-sodium-calcium-fluoro-phosphoro-silicate (18:9:8:16:3:46) 0.84g -Polyacrylic acid (MW 30000-45000) 0.15g	**Powder †**	*Alumino-silicate glass* (99%)
	Liquid	Distilled/deionized water	**Liquid †**	*2-hydroxyethyl methacrylate* (30-35%) *Polyacrylic acid* (20-30%) *Urethane Dimethacrlate* (<10%) *Distilled water* (20-30%)
Colour	L 2		A 2	
Batch number	1110001332		1108031	

† derived from manufacture material safety data sheet (2007)

- **PREPARATION OF MATERIAL SPECIMENS**

To make the dental material specimens Polytetrafluoroethylene (PTFE) moulds were prepared at the Dental School workshop at University of Dundee, with an inner diameter of 12 mm, and a thickness of 2 mm (Figure 3-1). PTFE rings were washed thoroughly with water and detergent, and then sterilized using Ultraviolet light for 24 hours before use.

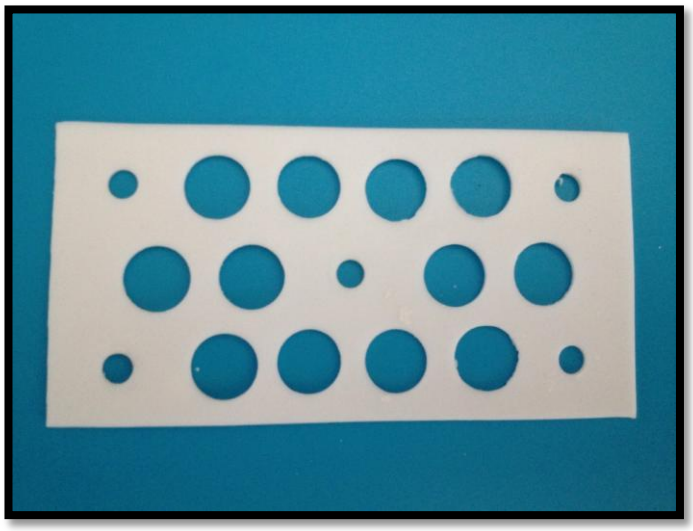


Figure 3-1 PTFE moulds.

Preparation of disks was carried out under sterile condition using sterilized instruments. Scoops, droppers and plastic spatulas provided by the manufacturers were rinsed thoroughly in 70% ethanol, and left to dry under Ultraviolet light for 24 hours before use. All baseline specimens of both commercial types of glass ionomer cements (ChemFil Superior and Fuji VIII $_{GP)}$ were mixed according to manufacturers' instructions and condensed using plastic instruments, into the PTFE mould which was placed on a PTFE sheet and then the PTFE mould was covered with another PTFE sheet before they were all screwed in between two metal plates. After setting the disks were removed from the mould by gentle hand

pressure, after unscrewed the metal plates. Then 60ml dishes were opened, inside the cabinet and the set disks which, made from glass ionomer cements were glued to the centre of the dish using superglue (SHERAMEGA 200, Espohsrabe 53, Lemforde, Germany) (figure 3-2). Once all the specimens were glued, the dishes were labelled and placed in the microbiological hood under UV light overnight (lid removed) for sterilization. The specimens were washed over the next 24 hours with Hanks balanced salt solution then left in serum free medium (SF-MEM) overnight.

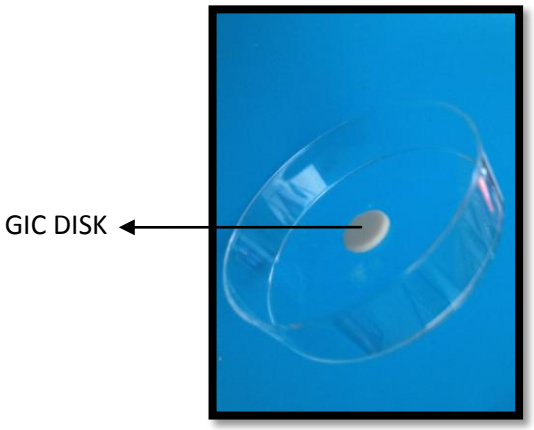


Figure 3-2 Glass ionomer disk attached to the dish.

- **CELL CULTURE MATERIALS**

- 70% ethanol in distilled water.
- MM1 (Normal Oral mucosa fibroblast cells).
- Foetal Calf Serum (SIGMA # D-5859, Sigma-Aldrich Company Ltd. Dorset, England).
- EGTA (SIGMA # E-8145, Sigma-Aldrich Company Ltd. Dorset, England).
- L-Glutamine (SIGMA # G-7513, Sigma-Aldrich Company Ltd. Dorset, England).
- Minimum Essential Medium Eagle (MEM) (SIGMA # M-0275, Sigma-Aldrich Company Ltd. Dorset, England).
- Penicillin-Streptomycin stabilized (SIGMA # P-4333, Sigma-Aldrich Company Ltd. Dorset, England).
- Trypsin/EDTA (SIGMA # T-4549, Sigma-Aldrich Company Ltd. Dorset, England).
- PBS (Phosphate buffered saline) (SIGMA # P-4417, Sigma-Aldrich Company Ltd. Dorset, England)
- Hanks balanced salt solution (SIGMA # H-4641, Sigma-Aldrich Company Ltd. Dorset, England).
- DMSO (Dimethyl sulphoxide) (SIGMA # D-5859, Sigma-Aldrich Company Ltd. Dorset, England).

- **CELL CULTURE PROCEDURE**

Normal oral mucosa fibroblast cells (MM1) were cultured, in 90mm dishes with 6ml growth medium each and incubated at 37°C in a CO_2 incubator with 5% CO_2 until confluent. They were routinely passaged by trypsinization. In brief, 90mm dishes were opened, inside the cabinet, the growth medium was aspirated and the monolayer washed twice with 5ml Hanks balanced salt solution. Following this, 2ml of Trypsin/EDTA solution was added to each 90mm dish and left for 5 minute over a hot plate, until the cells had fully detached. Detachment was confirmed by viewing under a light microscope then, 2ml of fresh growth medium was added, and the cells were collected and transferred to a centrifuge tube using a disposable pipette. The cell suspension was then centrifuged for 5 minutes at 900 rpm, and the growth medium decanted. Fresh growth medium was then added and the cell pellet re-suspended. The cellular concentration was determined by counting using an automatic cell counter (Biorad). Cells were seeded in each 60mm dish around and over the specimens at a concentration of 5 X 10^5 cells/ml for 21 days at 37°C and 5% CO_2. The medium was changed every other day. The cells were monitored under the light microscope and pictures were taken to the cells located close and away from the specimens every 3 days (Figure 3-3).

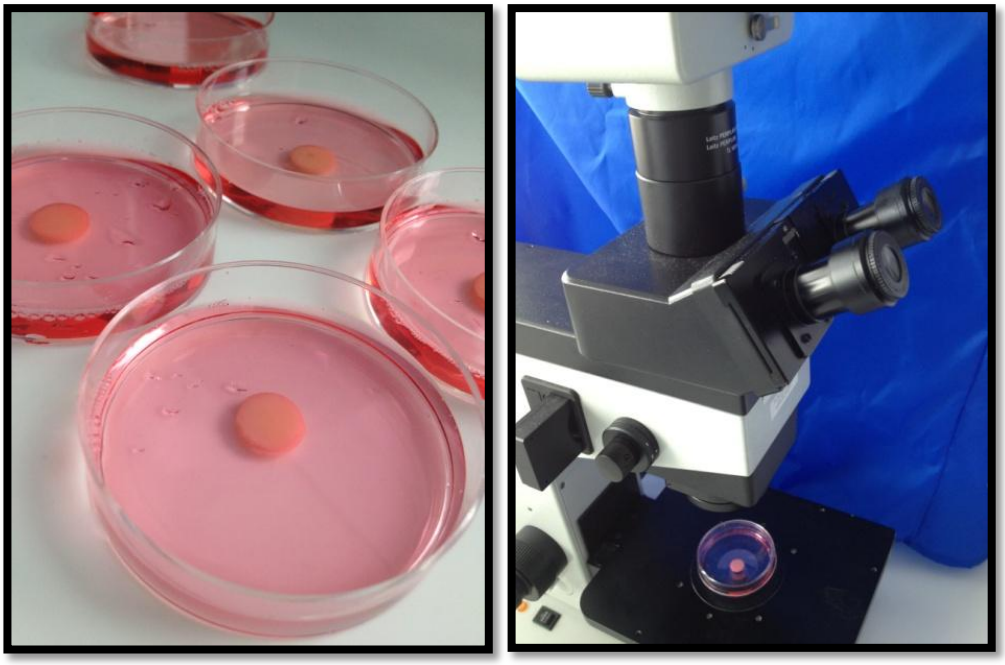


Figure 3-3 Cells were monitored under the light microscope.

- **VIABILITY SCORE**

Ten examiners experienced in cell biology were asked to rank the cells using visual observation method as is shown in Table 3-2 (ICCVAM, 2011)

Table 3-2 Visual Observation Scoring Table for Cell Viability.

VIABILITY SCORE	BRIEF DESCRIPTION
1	Normal cells Morphology and cell Density
2	Altered cell Morphology and/or small gaps between cells
3	Altered cell morphology and/or large gaps between cells
4	Few (or no) visible cells

- **STATISTICAL ANALYSIS**

The effect of the materials on cells was compared to that of the control using the scoring values as indicators of cell viability seen over all observation times. The data were analyzed using non-parametric one-way ANOVA (A Kruskal-Wallis test) ($P < 0.05$). Further analysis to localise significant difference was performed using Dunn's Multiple comparison test.

GraphPad PRISM software (Version 5.0, GraphPad Software Inc, San Diego, CCalifornia, USA) was used for the statistical analysis.

3.2.1.1.2 DETERMINATION OF THE OPTIMAL BIOACTIVE ADDITIVES AND CONCENTRATION

ChemFil Superior glass ionomer was chosen to be modified as it showed better bicompatiality than Fuji VIII $_{GP.}$ Three different bioactive additions [1- Collagen I, 2- Stramuann® Emdogain, 3- Gly-Arg-Gly-Asp-Ser (RGD)] (Table 3-3) were added to ChemFil Superior glass ionomer in order to produce bioactive specimens.

Table 3-3 Bioactive additions.

MATERIALS	MANUFACTURE
Type I Collagen	University of Dundee lab (Schor, 1980)
Straumann® Emdogain	Institute Stramuann®,#CH-4002, Basel, Switzerland
Gly-Arg-Gly-Asp-Ser (RGD)	SIGMA-ALDRICH,# 3050 Spruce Street, St. Louis, MO 63103 USA

- Type I collagen was added to the mixing water of glass ionomer in two different weight ratios 10µg/ml and 100 µg /ml to form aqueous solutions of 0.1 % and 0.01% type I collagen.
- RGD was added to glass ionomer mixing water with two different concentration 1mg/ml and 5mg/ml.
- Emdogain was added to the mixing water of glass ionomer in two different concentration of 4mg/ml and 8mg/ml

All bioactive additives were incorporated into the material and mixed according to manufactures instruction at a powder: liquid ratio of 1: 1 and mixed using plastic instruments, into the PTFE mould which was placed on a PTFE sheet and then the PTFE mould was covered with another PTFE sheet before they were all screwed in

between two metal plates. After setting the disks were removed from the mould by gentle hand pressure after being unscrewed from the metal plates. 60mm dishes were opened inside the sterile cabinet and the set disks made from both bio-modified at deferent concentration and unmodified ChemFil Superior (control) were glued inside the dishes and labelled. The dishes were placed in the microbiological hood under UV light overnight (lid removed) for sterilization. Then the specimens were washed over the next 24 hours with Hanks balanced salt solution then left in SF-MEM overnight.

CELL CULTURE AND VIABILITY SCORE

Normal oral mucosa fibroblasts were cultured around and over the specimens and monitored and ranked the same way as described in the previous section (3.2.1.1DETERMINATION OF THE OPTIMAL MATERIALS FOR ATTACHMENT OF CELLS).

STATISTICAL ANALYSIS

The effect of the materials on cells was compared to that of the control using the scoring values as indicators of cell viability seen over all observation times. The data were analyzed using non-parametric one-way ANOVA (A Kruskal-Wallis test) (α=0.05). Further analysis to localise significant difference was performed using Dunn's Multiple comparison test.

GraphPad PRISM software (version 5.0, GraphPad Software Inc, San Diego, California, USA) was used for the statistical analysis.

3.2.1.2 *CELL VIABILITY TESTING (MTT ASSAY)*

The MTT assay is a colorimetric assay for measuring cellular viability the assay measures the conversion tetrazolium dye (3-[4,5-dimethylthiazol]-2-yl-2,5-diphenyl tetrazolium bromide, or MTT), by the mitochondria of living cells to into insoluble formazan crystals (Mosmann, 1983), which can be quantified after solubilization using a spectrophotometer (Givens et al., 1990) .

- **MATERIALS**
    - 70% ethanol in distilled water.
    - MM1 (Normal Oral mucosa fibroblast cells).
    - Foetal Calf Serum (SIGMA # D-5859, Sigma-Aldrich Company Ltd. Dorset, England).
    - EGTA (SIGMA # E-8145, Sigma-Aldrich Company Ltd. Dorset, England).
    - L-Glutamine (SIGMA # G-7513, Sigma-Aldrich Company Ltd. Dorset, England).
    - Minimum Essential Medium Eagle (MEM) (SIGMA # M-0275, Sigma-Aldrich Company Ltd. Dorset, England).
    - Penicillin-Streptomycin stabilized (SIGMA # P-4333, Sigma-Aldrich Company Ltd. Dorset, England).
    - Trypsin/EDTA (SIGMA # T-4549, Sigma-Aldrich Company Ltd. Dorset, England).
    - PBS (Phosphate buffered saline) (SIGMA # P-4417, Sigma-Aldrich Company Ltd. Dorset, England)
    - Hanks balanced salt solution (SIGMA # H-4641, Sigma-Aldrich Company Ltd. Dorset, England).

- DMSO (Dimethyl sulphoxide) (SIGMA # D-5859, Sigma-Aldrich Company Ltd. Dorset, England).
- MTT (3-(4,Dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) (SIGMA # M-2128, Sigma-Aldrich Company Ltd. Dorset, England).

3.2.1.2.1 DETERMINATION OF THE OPTIMAL MATERIALS FOR ATTACHMENT OF CELLS USING MTT ASSAY

- **SPECIMENS PREPARATION**

All baseline specimens of both commercial types of glass ionomer cements (ChemFil Superior and Fuji VIII $_{GP}$) were prepared on the same way as described in the previous experiment section (3.2.1.1 DETERMINATION OF THE OPTIMAL MATERIALS FOR ATTACHMENT OF CELLS).

48 well plates were opened, inside the cabinet and the set disks which, made from both materials (ChemFil Superior and Fuji VIII $_{GP}$) were placed inside the wells. Once all the disks were at the bottom of the wells the 48 well plates were placed in the microbiological hood under UV light overnight (lid removed) for sterilization. After specimens were washed over the next 24 hours with Hanks balanced salt solution then left in SF-MEM overnight.

Cell viability testing (MTT ASSAY)

Normal oral mucosa fibroblast cells (MM1) were cultured , in 90 mm dishes with 6ml growth medium each, and incubated at 37 ˚C in a CO_2 incubator (Thermo scientific Hera Cell 240) with 5% CO_2 until confluent. They were routinely passaged by trypsinization.

In brief, 90mm dishes were opened, inside the cabinet (Class II Microbiological Safety Cabinet 1, BioMAT Medical Air Technology**)**, the growth medium was aspirated and the monolayer washed twice with 5 ml Hanks balanced salt solution. Following this, 2 ml of Trypsin/EDTA solution was added to each 90mm dish and left for 5 minute over a hot plate, until the cells had fully detached. Detachment

was confirmed by viewing under a light microscope then, 2 ml of fresh growth medium were added, and the cells were collected and transferred to a centrifuge tube using a disposable pipette. The cell suspension was then centrifuged for 5 minutes at 900 rpm, and the growth medium decanted. Fresh growth medium was then added and the cell pellet re-suspended. The cellular concentration was determined by counting using an automatic cell counter (Biorad). Cells were seeded in two 48-well culture plates at a concentration of 5 X 10^4cells/ml for 24 hours and 72 hours at 37 ˚C and 5% CO_2. A pilot study was performed to determine the optimal cell density for the experiment. Cells at different density ($1x10^4$, $2x10^4$, $5x10^4$ cells/ml) were seeded in 48-well culture plates for 24 and 72 hours at 37˚C and 5% CO_2. The density of $5x10^4$ cells/ml was found to be optimal regarding to the confluence of the cells and was used in this study.

After 24 hours of incubation, the cell attachment, morphology and confluency of the wells were checked for both plates and 250µL of fresh growth medium was added to each well of the second plate and it was then returned to the incubator for another 48 hours at 37 ˚C and 5% CO_2. The medium was removed from the wells of the first plate by aspiration and the wells were then washed twice very gently with serum free medium MEM (SF MEM). 250µL MTT solution was added to each well then the plate was incubated at 37°C, 5% CO_2 for 3 hours. After, the MTT solution was removed from the wells by gently tapping the contents onto paper tissues and 250µL of DMSO was added to each well and the plate was placed on the shaker for 20 minutes at room temperature. Finally the resulting coloured solution was assessed by reading the plate spectrophotometrically on the Fluorostar Optima plate reader (BMG LABTECH) at a wavelength of 540nm.

The same procedure was repeated with the second plate after 72 hours of incubation at 37°C, 5% CO_2. The concentration of $4x10^4$ cells/ml was found to be optimal regarding the confluence of the cells and was therefore used in this study.

- **Statistical analysis**

Absorbance values obtained for each well represent the amount of MTT reduction, which is proportional to the number of viable cells. In order to assess the percentage of viable cells present in each well, the absorbance values were related to those of the control. This was achieved by setting the mean absorbance of the control at 100%.

$$Percentage\ of\ viable\ cells = \frac{\text{Absorbance value}}{\text{Mean Absorbance of the control}}\ \text{X } 100$$

GraphPad PRISM software (version 5.0, GraphPad Software Inc, San Diego, California, USA) was used for the statistical analysis.

The effects of the materials on the cells were compared to each other using the percent viability values as indicators of cell numbers, and the data were analyzed to determine the effect of type of materials on cell viability the data was analyzed by unpaired t test ($P < 0.05$).

3.2.1.2.2 DETERMINATION OF OPTIMAL BIOACTIVE ADDITIVES AND CONCENTRATION

- **Specimens preparation**

ChemFil Superior glass ionomer was chosen to be modified with three different bioactive additions and specimens prepared in the same way previous described in section (3.2.1.1.2 DETERMINATION OF THE OPTIMAL BIOACTIVE ADDITIVES AND CONCENTRATION).

- **Cell viability testing (MTT ASSAY)**

The MTT Assay was carried out on the same way as was described in previous section (3.2.1.2.1 DETERMINATION OF THE OPTIMAL BIOACTIVE ADDITIVES AND CONCENTRATION).

- **Statistical analysis**

GraphPad PRISM software (version 5.0, GraphPad Software Inc, San Diego, California, USA) was used for the statistical analysis.

This data was subject to analysis of variance with *post hoc* testing using the Tukey comparison of means test.

3.2.1.3 *IMMUNOCYTOCHEMISTRY (ICC)*

The objective is to label MM1 fixed in situ with primary antibody against Vimentin and visualisation by labelling with a fluorescent secondary antibody.

- **MATERIALS AND METHODS**

The normal oral mucosa fibroblast cell line (MM1) was cultured along with glass polyalkenoate and bio-modified glass polyalkenoate cements at an initial density of 0.5×10^6 cells per 60mm dish for 3 weeks. After, medium was aspirated from the dishes and the cells were washed 3 times using phosphate-buffered saline (PBS). Cells were then fixed with methanol for 20 minutes at room temperature, washed with 0.2% Triton X-100 in PBS for 5 minutes, then washed again 3 times with PBS, (5 minutes each), and then blocked with 5% normal goat serum (NGS) in PBS-T (PBS+0.1% Tween 20). After 60 minutes, the blocking solution was aspirated cells were washed with PBS three times 5 minutes each, and then, incubated overnight at +4°C with Vimentin R28 rabbit monoclonal antibody (# 3932S, Cell Signalling Technology). The antibody was diluted in 5% NGS in PBS-T dilutions used were 1:100 and secondary only. All dishes were rinsed gently with PBS, and then washed twice with PBS-T and 1 time with PBS for 5 minutes each. The cells were incubated with secondary antibody Anti-Rabbit IgG conjugated with Alexa Fluor 488 (#4412, Cell Signaling Technology) diluted 1:1000 in 5% NGS in PBS-T for 2 hours. Finally, all dishes were rinsed gently with PBS, and then washed twice with PBS-T and then with PBS for 5 minutes each. The overslips were mounted using aqueous mount (Sigma) Images were acquired using an inverted fluorescent microscope (Olympus Model IMT-2) equipped with a digital camera and the image acquisition software (Metamorph software).

3.2.1.4 *PROTEIN BIOCHEMISTRY*

3.2.1.4.1 CELL LYSIS

Normal oral mucosa fibroblast cell line (MM1) was cultured along with glass polyalkenoate and bio-modified glass polyalkenoate cements at an initial density of 0.5 $\times 10^6$ cells per 60mm dishes. After 3 weeks in culture the medium was aspirated from the dishes and the cells were washed 3 times using phosphate-buffered saline (PBS). Total cell protein was then harvested using a cell lysis buffer (50mM Tris HCl pH 7.2-tris (hydroxymethyl) aminomethane, 150 mm sodium hydrochloride, 0.1% (W/V) SDS-Sodium dodecyl sulfate (anionic surfactant), 1.0% (V/V) Triton, 1.0% (E/V) sodium deoxy and 5mM and 5Mm EDTA) containing protease inhibitors (# 04693132001, Roche Diagnostics, Burgess hill, UK). 500 µl of the lysis buffer were added to each dish and the dishes were then incubated on ice for 10 minutes; finally, each dish was scraped and the lysates were then collected in Eppendorf tubes, appropriately labelled, and stored at -20°C.

3.2.1.4.2 SDS PAGE (POLYACRYLAMIDE GEL ELECTROPHPRESIS)

SDS PAGE is a common technique used to separate proteins according to their size.

The frozen lysates were thawed and then spun at 13000rpm for 5 minutes. Samples were combined with equal volume of Laemmli loading buffer (62.5 mM Tris HCl pH 6.8, 2% (w/v) SDS, 25% (v/v) Glycerol, 0.01% (w/v) Bromophenol Blue, 5% (v/v) 2-Meercaptoethanol) and were heated at 95°C for 5 minutes, prior to loading onto the gel (Bio Rad Any KD Stain free Tax pre –cast gel). 5 µL cell lysate per well were loaded, and 3 µL of Magicmark XP (invitrogen Ltd, Paisely UK) was loaded as well, for molecular weight estimation using a Gilson pipette. Gels were run at a constant voltage of 110 -150 volts in running buffer (25 mM Tris HCl

pH 8.3, 192 mM glycine, and 0.1% (w/v) SDS). Continue electrophoresis until the dye front (blue line) reaches the black line at the bottom of the gel cassette. Then the gel was removed gently from the cassette and placed the gel to be western blotted into transfer buffer.

3.2.1.4.3 WESTERN BLOTTING

Western blotting is a method used to transfer the protein bands, obtained by SDS PAGE fractionation of the cell lysates, onto a nitrocellulose membrane using an electrical current and to subsequently probe the membrane with a primary antibody that specifically binds to the target protein (Vimentin).

- ***MATERIALS***

- Transfer Buffer: (5.82g Tris, 2.93g Glycine, 3.75ml 10% (w/v) SDS, 200ml Methanol made up to 1 litre with dH_2O).
- TBST: (24.2 Tris; 80g Sodium chloride ; 5ml Tween; 14ml Hydrochloric acid pH 7.6; 1 litre dH_2O)
- Blocking Buffer (100ml 1x Tris-buffered saline Tween TBST (PH 7.6); 1g dried milk powder.
- TBS: TBST without Tween 20
- Bio Rad Extra thick blotting paper
- Nitrocellulose membrane (0.2µm; Bio-Rad)
- Bio-Rad Trans-Blot SD Semi-Dry Electrophoretic cell
- BioRad Immun- Star Western C
- Antibodies

Primary antibody

Anti-vimentin (#3932, Cell Signalling, Cell Signalling technology, Wilbury Way, UK)

Secondary antibody

Anti-rabbit IgG (#7074, Cell Signalling, Cell Signalling technology, Wilbury Way, UK)

- ***METHODS***

The sample proteins were transferred from the gel to nitrocellulose membrane. The gels were trimmed and then rinsed in the transfer buffer. The Western blot was prepared by placing a piece of BioRad extra thick paper soaked in transfer buffer onto the electrode, followed by a piece of Nitrocellulose membrane, the gel then covered by another piece of soaked BioRad extra tick paper. The Sample proteins were then transferred to the Nitrocellulose membrane at 15v for 42 minutes. After blotting, the membranes were blocked in 1% (w/v) milk TBST for 10 minutes and then exposed to primary antibody overnight (Anti-vimentin (Cell Signalling #3932) diluted 1:2000 in 1% (w/v) milk TBST). Followed by washing 3 times with TBS-T for 20 minutes each wash. Next, the membrane was incubated with secondary antibody from Cell Signalling (Anti-rabbit IgG –HRO labelled product no. 7074, Diluted 1:2,000 in 1% (w/v) milk TBST) for an hour. Membranes were then washed again with TBST 3 time for 20 minutes each, incubated with SuperSignal® West Pico chemilumniscent substrate (Thermo Scientific) for 1-5 minutes. Finally, the chemiluminescence was detected and documented by using a BioRad gel doc system.

In order to assess the percentage of Vimentin expression by cells cultured with materials, the band density were related to those of the control (MM1 + ChemFil superior). This was achieved by setting the density of the control at 100%.

$$Percentage\ of\ vimentin\ expressed\ by\ cells = \frac{\text{Band density of each material}}{\text{Band density of the control}} \text{ X } 100$$

3.2.2 DETERMINATION OF THE PHYSICAL PROPERTIES OF THE GLASS POLYALKENOATE CEMENTS AT BASELINE AND FOLLOWING BIOACTIVE ADDITIONS

At baseline and following the additions (additions identified in this work as having potential for cellular attachment) the following physical properties, of potential significance for clinical durability, were determined.

- Surface hardness (5 specimens)
- Compressive strength (15 specimens)
- Diametral compressive strength (15 specimens)
- 3 point flexural strength (15 specimens)
- Diametral compressive fatigue limit (15 specimens)
- Biaxial flexural strength (10 specimens)
- Adhesive shear bond strength to bovine enamel (15 specimens)

3.2.2.1 *SPECIMEN FABRICATION AND STORAGE*

All baseline specimens were proportioned and mixed according to the manufacturer's instructions. In the case of the bioactive additions; (1- Collagen TypeI, 2- Gly-Arg-Gly-Asp-Ser (RGD), 3- Stramuann® Emdogain). These were added according to the methods described below. Such addition was identified from the cellular work of this thesis (3.2.1 INVESTIGATION OF BIOACTIVE ADDITIONS TO GLASS POLYALKENOATE CEMENTS) as offering greatest potential to foster cellular interaction. In all bioactive addition the material was dispersed according to the manufactures instructions at a powder: liquid ratio of 1: 1 the additions investigated for this part of the work were.

a. **Collagen type I 0.1% (100µg/ml)**
b. **RGD (5mg/ml).**

Once fabricated all specimens were stored in distilled water at 37 °C for one week prior to testing.

(a) Fabrication of specimens used for compressive and flexural testing.

A variety of moulds were used to manufacture the specimens. In the case of flexural specimens a sectional Perspex mould, giving a specimen size of 25 x 2 x 2 mm was used (Figure 3.4).

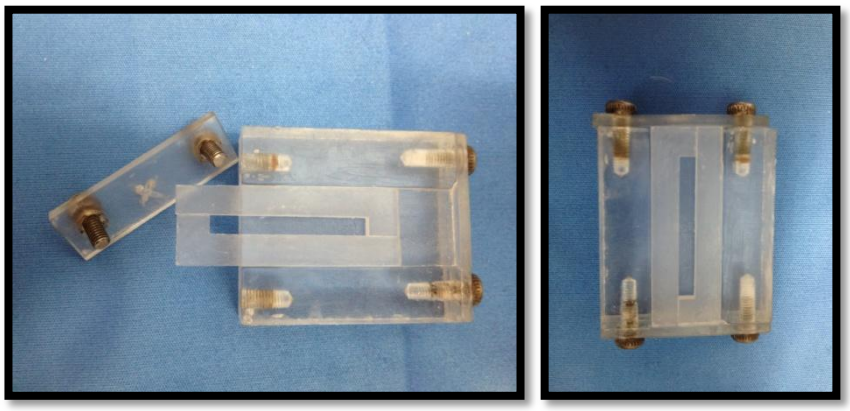


Figure 3-4 The Perspex mould used to flexural specimens.

For the specimens tested for compressive strength, diametral compressive strength and diametral compressive fatigue a split stainless steel mould, giving specimens sizes of 6 mm long and 4 mm diameter was used (Figure 3-5).

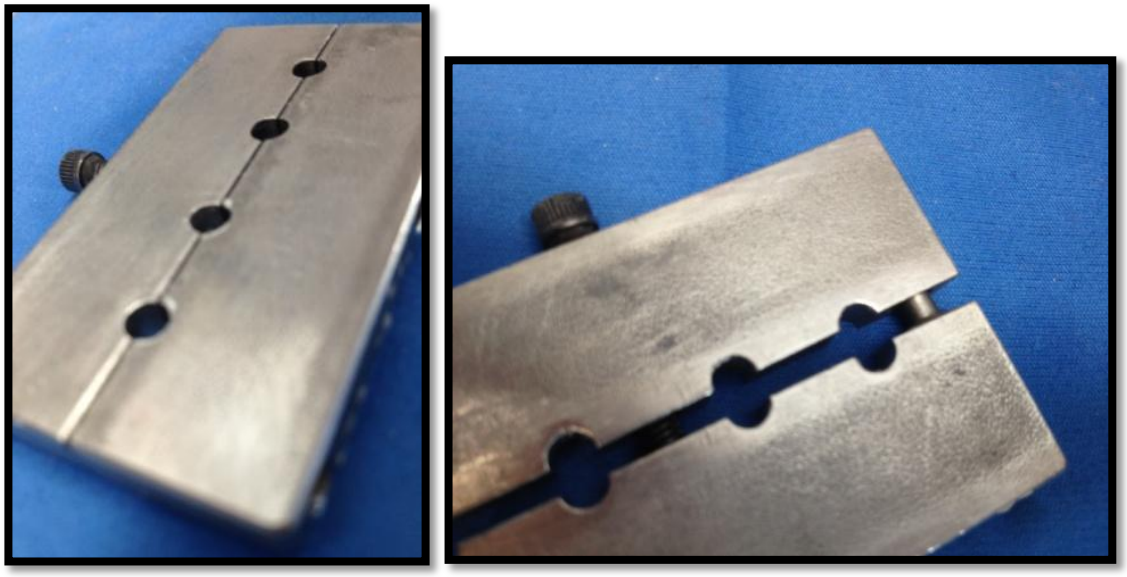


Figure 3-5 The Split stainless steel mould used to fabricate compressive, diametral compressive and compressive fatigue specimens.

Biaxial flexural strength specimens were fabricated in silicone rubber moulds giving specimen sizes of 2 mm thick X 12 mm diameter (Figure 3.6).

None of the moulds was vaselined, to facilitate specimen release, other than the stainless steel compressive mould.

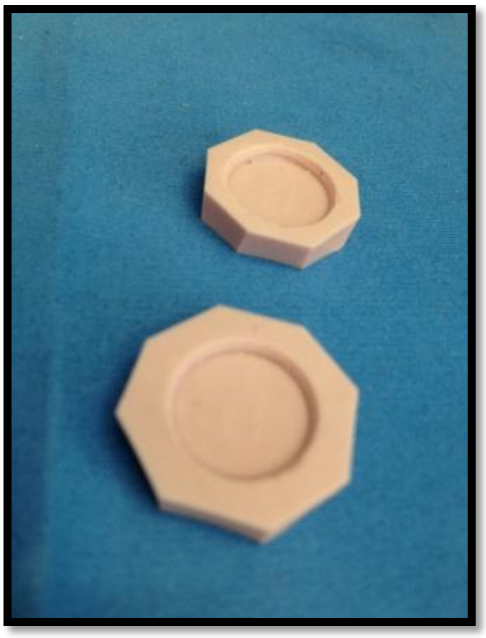


Figure 3-6 The silicone rubber moulds used to fabricate biaxial flexural strength, surface hardness, and adhesive shear bond strength specimens.
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Prior to mixing the cements under test the compressive strength mould was placed upon a flat glass slab covered by a clear cellulose matrix strip (Hawe-Neos Dental CH-6934 Bioggio Switzerland). In all other cases the mould designs themselves contained a flat base against which the base of the specimen was formed. Thereafter the mixed cement was applied into the well of the moulds using a plastic spatula, with packing action, to slight excess. A cellulose matrix strip was then applied to the exposed surface and pressure applied to the material through a flat glass slab on which was placed a 5 Kg weight for 5 minutes. Once this time had elapsed the specimen was removed from the mould. If upon visual inspection no defects were found the specimen was accepted for storage and testing.

(b) Fabrication of specimens for hardness testing

Hardness testing specimens were fabricated in a circular silicone rubber moulds giving specimen sizes of 2 mm X thick 12 mm diameter (figure 3-6) to produce 20 discs of glass ionomer cements in total comprises of unmodified ChemFil Superior and GC Fuji VIII and modified ChemFil Superior (with RGD and also collagen Type I). For each state 5 specimens were made and following a period of 5 minutes within the mould the specimens were ejected and stored for one week in distilled water at 37 C° prior testing.

The mixed cement was applied into the well of the moulds using a plastic spatula, with packing action, to slight excess. A cellulose matrix strip was then applied to the exposed surface and pressure applied to the material through a flat glass slab on which was placed a 5kg weight for 5 minutes. Once this time had elapsed the specimen was removed from the mould. If upon visual inspection no defects were found the specimen was accepted for storage and testing.

(c) Fabrication of specimens for bond strength testing

24 bovine molar teeth, with their roots removed and pulps extirpated, were sectioned longitudinally and mounted in circular epoxy resin blocks ((Bonda Clear Casting Resin, Bondaglass Vost Ltd, Kent, UK)) with their buccal/palatal surfaces upper most. The exposed surfaces were rendered flat flush with the surrounding mounting epoxy resin using a PM5 precision lapping and polishing machine (Longitech, Glasgow, Scotland) and a slurry of calcined Aluminium Oxide powder with a particle size of 9 μm (Longitech, Glasgow, Scotland), for subsequent cement application and testing.

Prior to cement application all prepared samples of tooth were stored in a incubator, at 37 °C, in distilled water for one month prior to cement application. This was in an endeavour to ensure uniform inter specimen hydration. Thereafter the specimens were removed from storage and a circular washer (5mm diameter x 1.5 mm deep) was placed upon the exposed tooth surface. Through this the mixed glass polyalkenoate cement was applied, using a flat plastic instrument, and once

clinically set the washer was removed. Thereafter the completed specimen was stored in distilled water at 37°C for one week prior to testing.

3.2.2.2 ***SPECIMEN TESTING***

An Instron Universal testing machine (Model 4469, Instron Ltd., High Wycombe, UK) (figure 3-7) was used to perform all tests unless stated otherwise. The testing procedures described in this thesis utilised standard methodology used in the laboratories where the tests were undertaken.

Figure 3-7 An Instron Universal testing machine.

3.2.2.2.1 SURFACE HARDNESS

A type D Shore Durometer (Shore Instrument and manufacturing Co, Jamaica, New York, USA) was used to measure this property (Figure 3-8). Prior to its use its calibration was checked against its supplied calibration and each sample was subjected to one indentation, yielding a shore hardness value.

For each state and material hardness values were expressed as a mean and standard deviation of five samples, measured once each.

Figure 3-8 Shore Instrument.

3.2.2.2.2 COMPRESSIVE STRENGTH

This was determined at a cross head speed of 1 mm min^{-1}. Prior to testing the length and diameter of each specimen was measured using a micrometer. The results for each material and state were expressed as a mean and standard deviation. This data was subject to analysis of variance with *post hoc* testing using the Tukey comparison of means test.

The formula used to determine compressive strength was:

$$\textbf{Compressive Strength (MPa)} = \frac{\textbf{Force at Failure in Newtons}}{\textbf{Cross Sectional Area of Specimen}}$$

3.2.2.2.3 DIAMETRAL COMPRESSIVE STRENGTH

This was determined at a cross head speed of 1 mm min^{-1}. Prior to testing the length and diameter of each specimen was measured using a micrometer. The results for each material and state were expressed as a mean and standard deviation. This data was subject to analysis of variance with *post hoc* testing using the Tukey comparison of means test. The formula used to determine compressive strength was:

$$\textbf{Diametral Compressive Strength (MPa)} = \frac{\mathbf{2F}}{\boldsymbol{\pi}\mathbf{DT}}$$

Where F is the force (N) at failure, D is the specimen diameter (mm) and T is its length.

3.2.2.2.4 THREE POINT FLEXURAL STRENGTH

This was determined at a cross head speed of 1 mm min^{-1}. Prior to testing the breadth and depth of each specimen was measured using a micrometer. A support separation distance of 20 mm was used for this test. The results for each material and state were expressed as a mean and standard deviation. This data was subject to analysis of variance with *post hoc* testing using the Tukey comparison of means test. The formula used to determine flexural strength was:

$$\textbf{Flexural Strength (MPa)} = \frac{\mathbf{3FL}}{\mathbf{2bd^2}}$$

Where F is the force (N) at failure, L is the support separation distance in mm, b is the specimen width in mm and d is its depth in mm.

3.2.2.2.5 DIAMETRAL COMPRESSIVE FATIGUE LIMIT

This was determined at a cross head speed of 10 mm min-1 under load control. Each specimen was subjected to cyclic diametral compressive loading to failure or survival at 1500 cycles. At the commencement of the test a load of 2 Newton's was applied to the specimen before the load was increased to the maximum applied load for the test. Two Newton's was therefore the minimum force applied to each specimen. This was undertaken to minimize the effect of any bounce of the specimen upon the platten of the testing machine that could occur if the minimum load was zero Newton's. The starting level was the applied load necessary to achieve 60% of the measured diametral compressive strength at 10 mm min^{-1}. Testing followed the staircase method described by Draughn (1979) and where a specimen survived the next test maximum load was increased by a predetermined load increment and where it failed the load was decreased by the same increment. Upon completion of the test, after typically 15 specimens had been tested, analysis of the data was based upon the least frequent event (failure or survival). The applied stress levels were arranged in descending rank order (highest to lowest (rank 0)) and the numbers of failures or survivals for each of these was calculated. For the entire test, for a given material and bioactive addition, the total number of failures or survivals was determined. These totals were given the letter N (total of least frequent event). The data was used to calculate A using:

∑((the rank number of the applied stress of least frequent event) * (the frequency of event occurrence at that stress level)).

Thereafter B was calculated according to the formula;

$\sum$ ((Rank number of applied stress of least frequent event) ^{2} $*$ (frequency of event occurrence at that stress level)).

X_o was assigned to the lowest stress level considered in the analysis and d represented the stress increment used in the test.

The mean fatigue limit X was calculated using this expression;

$$\mathbf{X = Xo + d\left(\frac{A}{N} \pm \frac{1}{2}\right)}$$

If the analysis was based on survival the positive sign was used but where based on failure the negative sign was used.

The standard deviation (S) of the fatigue limit was calculated using the equation;

$S = 1.62\ d\ (NB\text{-}A^2/N^2 + 0.029)$ in cases where $(NB\text{-}A^2)/N^2$ was larger than 0.3.

In cases however where $(NB\text{-}A^2)/N^2$ was less than 0.3 it was calculated using the equation **$S = 0.53\ d$** as advocated by Dixon and Mood (1948) and recommended, in such circumstances, by Draughn (1979).

3.2.2.2.6 BIAXIAL FLEXURAL STRENGTH

A Universal Testing Machine (Model 4469, Instron Ltd., High Wycombe, UK), at a crosshead speed of 1 mm min^{-1}, was used to determine the bi-axial flexural strength of the discs. Each disc was placed on three circumferentially arranged fixed ball bearings spaced every 120^0 around the perimeter of a circle of radius 4 mm on a specially constructed jig. Load, to fracture, was applied perpendicular to the specimen's surface, at its centre, through a stainless steel rod of radius 1 mm at the point of specimen contact. The load at fracture was recorded and for each specimen bi-axial flexural strength was calculated using the following formula as reported by Shetty et al., (1983).

$$\sigma = \frac{AP}{t^2}$$

$$A = 3/(4\pi)[2(1+v)\ln(a/\overset{\star}{r}_{\circ}) + (1-v)\left\{\frac{2a^2-\overset{\star}{r}^2_{\circ}}{2b^2}\right\} + 1 + v]$$

Where P is the maximum at failure, v is Poission's ratio, a is the radius of the support circle (4 mm), b is the radius of disc specimen (6 mm), t is the thickness of the disc specimen (2 mm) and $r_{\circ}$ is the radius of the ball used on the loading surface. For small $r_{\circ}$ values such as that in the study:

$$r_{\circ} = \sqrt{(1.6r^2_{\circ} + t^2)} - 0.675t$$

Where $\overset{\star}{r}_{\circ}$ is an equivalent radius of contact between the loading ball and disc specimen, where loading can be considered to be uniform. The strength values were calculated using a Poisson's ratio 0.35 for the materials used to apply experimentally to materials of this type (Akinmade and Nicholson, 1993a).

3.2.2.2.7 ADHESIVE SHEAR BOND STRENGTH TO BOVINE ENAMEL

A specially constructed jig (Figure 3-9) mounted upon the load cell of the Instron Universal Testing Machine was used to determine the shear bond strengths of the Glass Polyalkenoate Cements to bovine tooth substance in both manufactured and modified form. For each combination of materials a total of 15 specimens were tested to failure. The blade of the assembly was applied, as close as possible, to the cement/tooth interface at a crosshead speed of 0.5 mm min^{-1}.

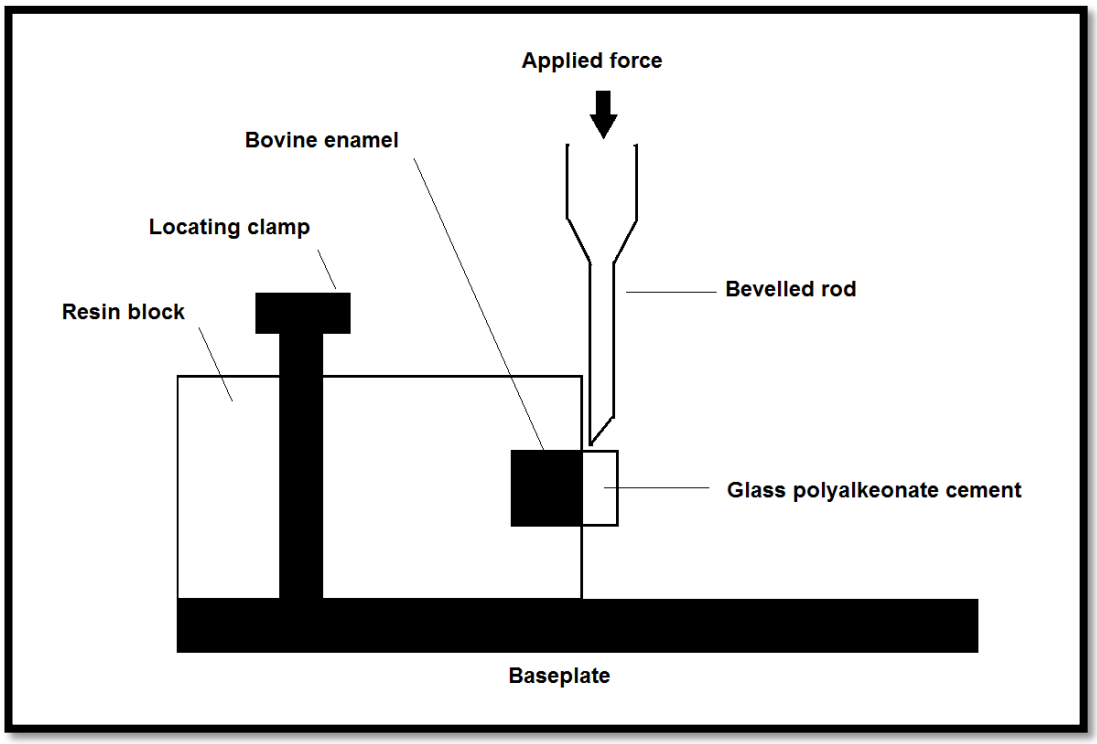


Figure 3-9 Schematic diagram of the adhesive shear strength testing.

The shear bond strength was calculated using the formula:

$$\textbf{Shear bond strength (MPa)} = \frac{\textbf{Force at Failure (N)}}{\textbf{Bonded Area (mm}^{\textbf{2}}\textbf{)}}$$

This data was subject to analysis of variance with *post hoc* testing using the Tukey comparison of means test.

4 RESULTS

4.1 THE QUESTIONNAIRE

In response to the invitation to participate in the questionnaire a total of 134 UK and 120 Libyan responses were received. These figures represent a percentage return rate of 34.25 % UK and 60 % Libya.

Due to changes in registration status of those invited to participate in the UK some questionnaires were returned as these individuals were no longer registered. This did not occur in Libya. Table 4-1 summarise the reasons given for return/non completion of the UK questionnaires as stated by the respondents.

Table 4-1 Reasons for Non completion /return of UK questionnaires.

Reasons	Number
Change in addresses	8
Not Specialty	4
Retirement	4
Others	1

Numbers in the body of the table represent the number responses

In relation to the UK responders the mean length of time they had been practicing dentistry was 20.64 years (SD= 11.31, Median= 20, minimum= 1, Maximum= 42).

In the case of Libyan respondents the mean length of time they had been practicing dentistry was 7.96 years (SD= 6.09, Median=6, minimum= 1, Maximum= 35)

Figures 4-1 and 4-2 graphically summaries the teeth most commonly affected by root surface caries in the experience of the respondents. It is evident that there is no clear difference between the countries in this respect.

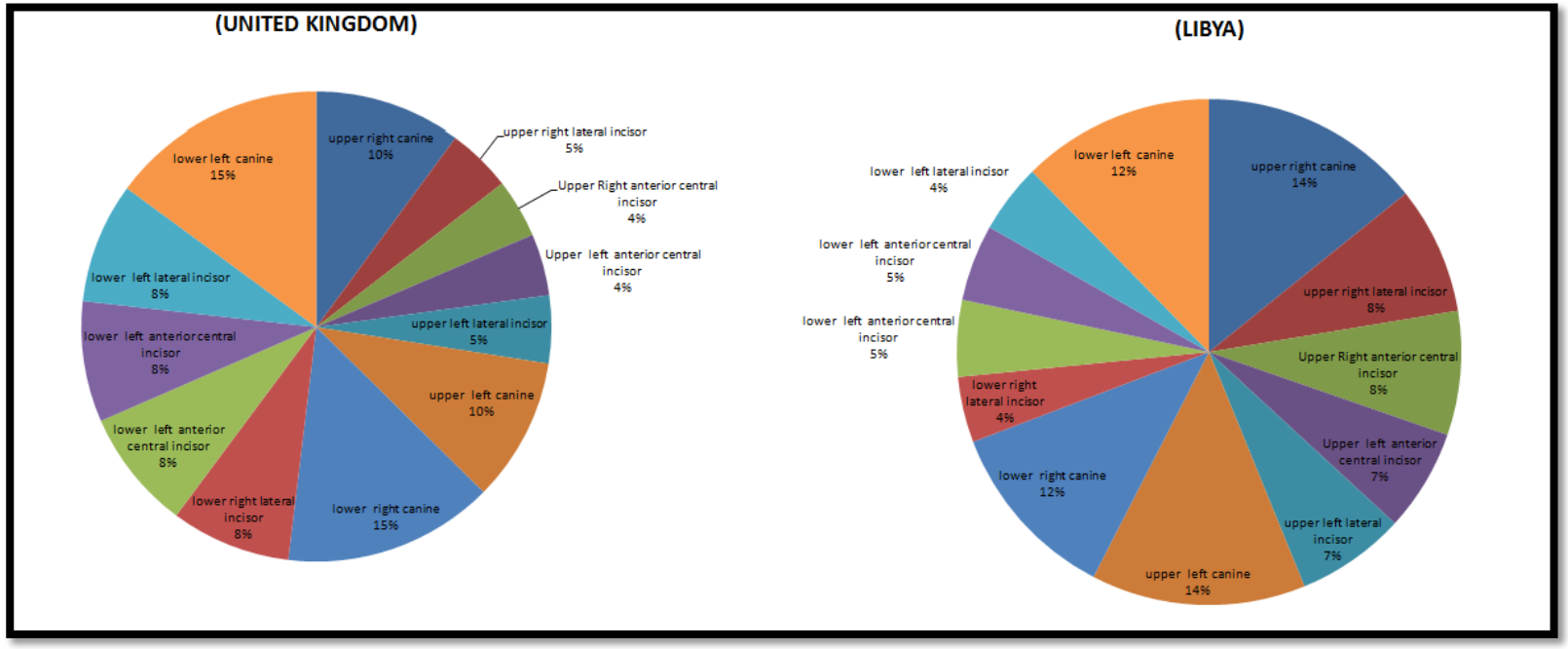


Figure 4-1 The anterior teeth most commonly affected by root surface caries according to the respondents of the questionnaire.

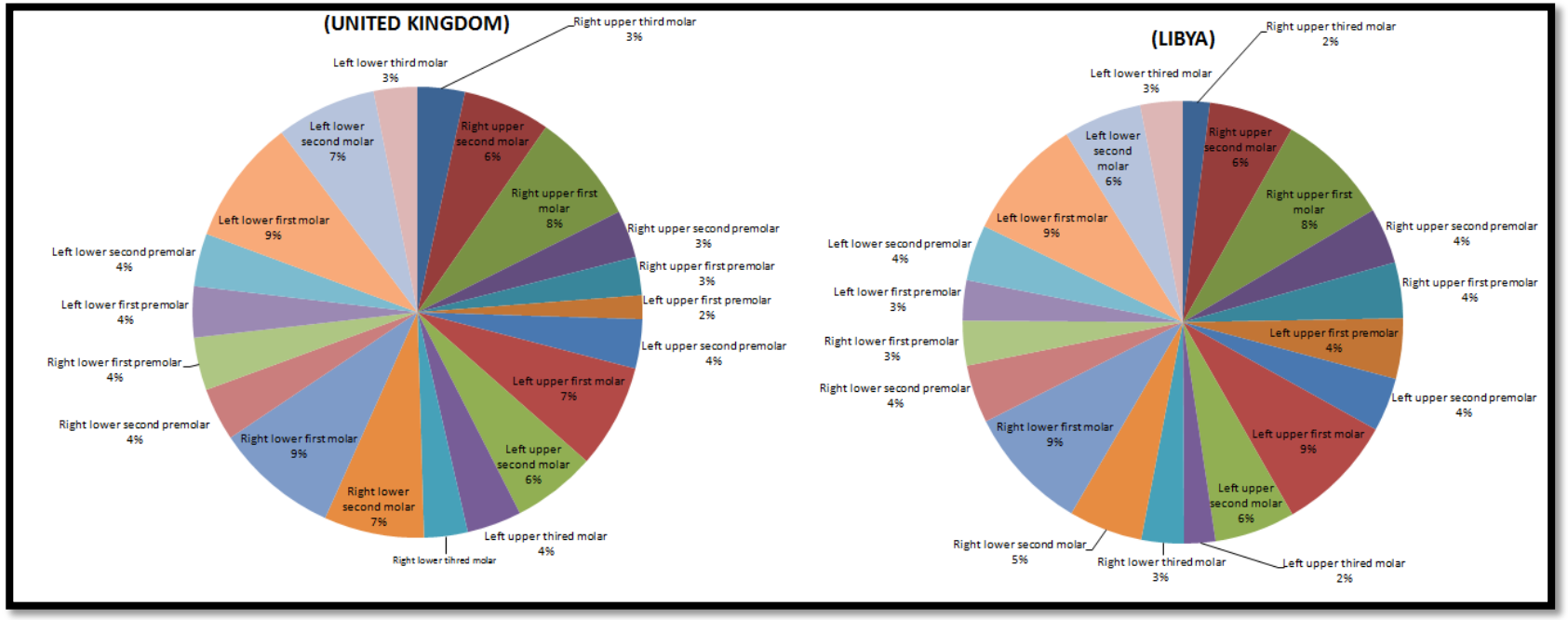


Figure 4-2 The posterior teeth most commonly affected by root surface caries.

Table 4-2 summarises the age groups thought to be most susceptible to the development of root surface caries according to both UK and Libyan respondents. A chi square test revealed a significant ($P < 0.01$) difference in the distribution of the responses from each country. Root caries in childhood was not reported by either country but a greater proportion of Libyan respondents reported root caries in adulthood (26.49% Libya of 2.25% UK).

Table 4-2 The age group most susceptible to root caries.

Age group	United kingdom		Libya	
Childhood	0	0.00%	0	0.00%
Adult	7	6.25%	31	26.49%
Elderly	105	93.75%	86	73.50%

Numbers in the table body are number of responses unless otherwise stated.

Where sex was taken into account (Table 4-3) a chi square test demonstrated significant differences in the responses of UK and Libyan respondents with the UK indicating proportionally more responses of no influence of sex upon root caries susceptibility. In Libya however a higher proportion of males were considered more likely to be susceptible to root caries. In both countries few indicated that females were susceptible.

Table 4-3 The most susceptible sex to root caries.

Gender	United Kingdom		Libya	
Males	29	25.22%	74	62.18%
Females	04	03.47%	08	06.62%
No difference between males and females	82	71.30%	37	31.09%

Numbers in the table body are number of responses unless otherwise stated

Table 4-4 summarises the perceived proportion of patients, in the experience of the UK and Libyan respondents, prone to root surface caries. This was proportionally greater for the UK (39.13%) compared to Libya (25.66%). Chi square testing confirmed statistically significant ($P < 0.05$) differences in the response of the two countries.

Table 4-4 proportion of patient prone to root surface caries in practices.

Does your practice have a large proportion of patient prone to root surface caries?	United kingdom		Libya	
Yes	45	39.13%	29	25..66%
No	70	60.86%	84	74.34%

Numbers in the table body are number of responses unless otherwise stated

Table 4-5 summarises the methods of detecting root surface caries stated by the respondents. Upon inspection of this it is clear that many permutations of the basic methods (visual, tactile, radiographs) were employed by the respondents. A chi square test reveled significant differences ($P < 0.05$) in the responses of the countries. In both countries the most common method of detecting root surface caries was visual. In Libya however radiographic detection was used more commonly than in the UK (34.86% Libya cf 22.03 UK).

Table 4-5 Stated methods of detection root surface caries.

Methods of detection	United Kingdom		Libya	
Visually	98	41.53%	65	37.14%
Tactile	80	33.90%	46	26.29%
Radiographs	52	22.03%	61	34.86%
Pain	0	02.54%	01	01.14%
Other techniques	6	00.00%	02	00.57%

Numbers in the table body are number of responses unless otherwise stated

Table 4-6 summarises the responses as to the most common surface affected by root surface caries. In both countries this was the buccal but in Libya proportionally more reports of root caries affecting the interproximal surface were encountered. Chi square analysis confirmed statistically significant ($P<0.05$) differences in the responses of the two countries.

Table 4-6 The surface most commonly affected by root surface caries.

Surface most commonly affected	United kingdom		Libya	
Labial (buccal) surface	68	67.33%	56	51.38%
Interproximal surface	27	26.73%	51	48.79%
Palatal (lingual) surface	6	5.94%	02	1.83%

Numbers in the table body are number of responses unless otherwise stated

Table 4-7 summaries the methods usually used to manage root surface caries. Chi square testing revealed a significant difference in the responses from both countries (*P<0.01*). In both a range of techniques were used but restoration was more commonly used in Libya (57.00% Libya versus 28.2% UK).

Table 4-7 The methods usually used to manage root surface caries.

Methods usually used to manage root surface caries	United kingdom		Libya	
Monitoring with prevention instruction	88	22.98%	27	15.00%
Dietary advice	88	22.98%	17	9.44%
Topical fluoride	90	23.50%	16	8.89%
Restoration	108	28.20%	103	57.22%
Ozone	3	0.78%	3	1.67%
Extraction	2	0.52%	12	6.67%
Oral hygiene instruction	3	0.78%	1	0.56%
Perio treatment	1	0.26%	1	0.56%
Others	0	0.00%	0	0.00%

Numbers in the table body are number of responses unless otherwise stated

Table 4-8 gives the factors cited by the respondents as influencing the selection of management method for root surface caries. Chi square testing demonstrated a significant (*P<0.01*) difference in the responses from the two countries. The diet of patients in the UK influenced to a greater extent the management of the root caries (UK= 23.86% versus Libya 08.11%)

Table 4-8 The factors that influence the selection of the management methods.

Factors that influence the selection of the management methods	United kingdom		Libya	
Patient oral hygiene	102	25.89%	74	33.33%
Diet	94	23.86%	18	8.11%
Patients age	59	14.97%	38	17.12%
Tooth type	23	5.84%	20	9.01%
Severity of the lesion	105	26.65%	70	31.53%
Socioeconomic	0	0.00%	1	0.45%
General health	4	1.01%	1	0.45%
Medication	2	0.51%	0	0.00%
Co-operation of patient	1	0.25%	0	0.00%
Patient dexenty	1	0.25%	0	0.00%
Attitude, special need, preference	1	0.25%	0	0.00%
Moisture control	1	0.25%	0	0.00%
Symptoms	1	0.25%	0	0.00%
Other	0	0.00%	0	0.00%

Numbers in the table body are number of responses unless otherwise stated

Table 4-9 gives the most commonly used restorative materials for treatment of root surface caries In both countries chi square testing revealed statistically significant differences in the responses (*P<0.05*) with proportionally more glass polyalkenoate cement being used in the UK (66.80% cf 47.02% Libya) and a greater amount of composite in Libya (26.49% cf 13.91% UK). Similar proportions of amalgam and compomer were used in both countries (approx. 10%)

Table 4-9 The most commonly used restorative material to restore root caries.

Most commonly used restorative material to restore root caries	United kingdom		Libya	
Amalgam	17	11.26%	14	10.07%
Glass Ionomer	101	66.89%	71	47.02%
Composite	21	13.91%	40	26.49%
Compomer	12	07.95%	14	09.27%
Others	0	00.0%	0	00.00%

Numbers in the table body are number of responses unless otherwise stated

Table 4-10 indicates the number of respondents reporting bleeding from the gingival tissues as a problem in restoring root surface caries. There was no statistically significant difference between the countries (*P > 0.05*) (chi square) with bleeding being reported as a problem both sometimes and frequently.

Table 4-10 Bleeding from the gingival tissues in restoring root surface caries.

Is bleeding from Gingival tissues problem in restoring root caries?	United kingdom		Libya	
Never	1	0.87%	12	10.61%
Sometimes	77	66.98%	55	48.67%
Frequently	37	32.17%	46	40.71%

Numbers in the table body are number of responses unless otherwise stated

Table 4-11 summarises the methods of finishing root surface caries restorations as reported by the respondents. Chi square testing revealed a difference in response according to country (*P<0.001*). With rotary cutting instruments being used to finish the restoration in both countries with however a greater proportion of respondents in Libya deferring this to a recall visit rather than at placement. (Libya 25.00% cf. 08.92% UK). Finishing at the placement was more common in the UK (UK 53.50% cf. Libya 35.34%).

Table 4-11 Methods of finishing the restoration.

Methods of finishing	United kingdom		Libya	
Hand finishing (sharp knives or scalers) at placement visit	53	33.76%	37	31.90%
Rotary finishing at placement visit	84	53..50%	41	35.34%
Hand finishing (sharp knives or scalers) at recall visit	5	3.18%	8	9.90%
Rotary finishing at recall visit	14	8.92%	29	25.00%
Strips- fine sand plate	1	0.64%	0	0.00%
Seal restorative, scalar	0	0.00%	1	0.86%
Others	0	0.00%	0	0.00%

Numbers in the table body are number of responses unless otherwise stated

Tables 4-12 and 4-13 Summarises the reported follow up, and its interval, of root caries restorations as reported by the respondents.

Chi square testing revealed significant (*p<0.01*) differences between the countries. In the UK it was most common to follow up root caries restorations (UK 66.67% cf. Libya 29.52%) every six months (UK 80%).

Table 4-12 Follow up of patients with root caries restorations.

Follow up of patients	United kingdom		Libya	
Yes	74	66.67%	31	29.52%
No	15	13.50%	16	15.24%
Sometimes	22	19.82%	58	55.24%

Numbers in the table body are number of responses unless otherwise stated

Table 4-13 Following up intervals of patients with root surface caries restorations.

Following up intervals	United Kingdom		Libya	
1 to 4 months	11	15.71%	13	44.83%
6 Months	56	80.00%	15	51.73%
9 to12 months	3	4.29%	1	3.45%

Numbers in the table body are number of responses unless otherwise stated

Table 4-14 summarises the respondents impression of the average life span of root caries restorations, formed from the preferred material of the respondents. A chi square test of this data demonstrated significant difference between the countries.

Proportionately more root caries restoration failed in the first year in Libya (15.38% Libya cf. 0% UK). Longer survival times were reported in the UK.

Table 4-14 The average life span of the restoration most commonly used for restoration root surface caries.

Average life span	United kingdom		Libya	
Less than a year	0	0.00%	16	15.38%
1 to 5 years	85	78.70%	82	78.85%
More than 5 years	23	21.30%	6	5.77%

Numbers in the table body are number of responses unless otherwise stated

The table 4-15 summarises the lifestyle factors reported as being associated with the development of root surface caries. There was no significant difference in the responses of the two countries (Chi Square). In both countries giving up smoking was the factor most considered to give rise to root surface caries. Surprisingly diet was considered by the respondents to be relatively unimportant (UK 4.44%, Libya 0%)

Table 4-15 The lifestyle events associated with root surface caries development.

Lifestyle events associated with root surface caries	United kingdom		Libya	
Bereavement	34	18.89%	19	15.78%
Retirement	34	18.89%	26	26.49%
Giving up smoking	39	21.67%	54	44.63%
Loss of job	21	11.67%	14	11.57%
Oral hygiene	10	5.56%	4	3.31%
Alcohol	0	0.00%	2	1.63%
Smoking	0	0.00%	1	0.83%
General health condition	25	13.89%	1	0.83%
Diet	8	4.44%	0	0.00%
Partial denture	3	1.67%	0	0.00%
Age	6	3.33%	0	0.00%
Other	0	0.00%	0	0.00%

Numbers in the table body are number of responses unless otherwise stated

Table 4.16 and figures 4.3 to 4.21 summarise the rankings of importance of factors in the development of root surface caries according to the country of the respondents.

These did not statistically differ in the cases of numbers of teeth present (figure 4-3), presence of partial dentures (figure 4-4), oral hygiene state (figure 4-5), physical disability (figure 4-6), mental disability/senility (figure 4-7), consumption of fizzy drinks (figure 4-8), overhanging restoration (figure 4-9) and poor general health (figure 4-10). There was a statistically significant difference between the countries responses in the cases of Degree of crowding (figure 4-11), Cigarette smoking (figure 4-12), Total amount of sugars consumed (figure 4-13), Frequency of sugar intake (figure 4-14), Active periodontal disease (figure 4-15), Consumption of alcohol (figure 4-16), Poor crown margins (figure 4-17), Gingival recession (figure 4-18), Reduce salivary flow (figure 4-19), Presence of erosion (figure 4-20), Presence of abrasion cavity (figure 4-21).

Table 4-16 The factors are important in the development of root surface.

The factors are important in the development of root surface where **1** =very important ,**2**=quite important, **3**=fairly important, **4**=not at all important									
	United kingdom					**Libya**			
	1	**2**	**3**	**4**		**1**	**2**	**3**	**4**
Number of teeth present	11	32	29	39	NS	26	30	30	34
Degree of crowding	22	39	34	19	***	64	33	14	3
Presence of a partial denture	52	36	18	8	NS	36	39	24	12
Cigarette smoking	14	45	34	21	***	52	31	25	5
Total amount of sugars consumed	74	23	12	6	***	35	47	31	5
Frequency of sugar intake	96	12	2	6	***	48	42	19	4
Oral hygiene state	98	9	3	4	NS	100	5	4	2
Physical disability	65	30	14	3	NS	45	40	19	6
Mental disability/senility	64	35	10	3	NS	68	26	14	4
Active periodontal disease	39	43	22	7	***	79	20	7	6
Consumption of alcohol	11	24	50	24	***	49	40	16	5
Consumption of fizzy drink	47	34	19	12	***	16	40	37	14
Overhanging restoration	31	38	34	9	NS	46	33	24	10
Poor crown margins	39	38	28	8	**	60	31	16	2
Gingival recession	39	44	22	8	***	76	24	8	4
Reduce salivary flow	84	19	7	2	*	66	37	7	2
Presence of erosion	13	28	47	24	***	38	44	20	12
Presence of abrasion cavity	9	28	43	31	***	32	49	24	7
Poor general health	54	38	19	2	NS	49	40	14	10

Where significant, as identified by Chi square testing, level of significance is shown as NS= Not significant, *= P < 0.05, **= P < 0.01, *** = P<0.001. Numbers in the body of the table are number of respondents.

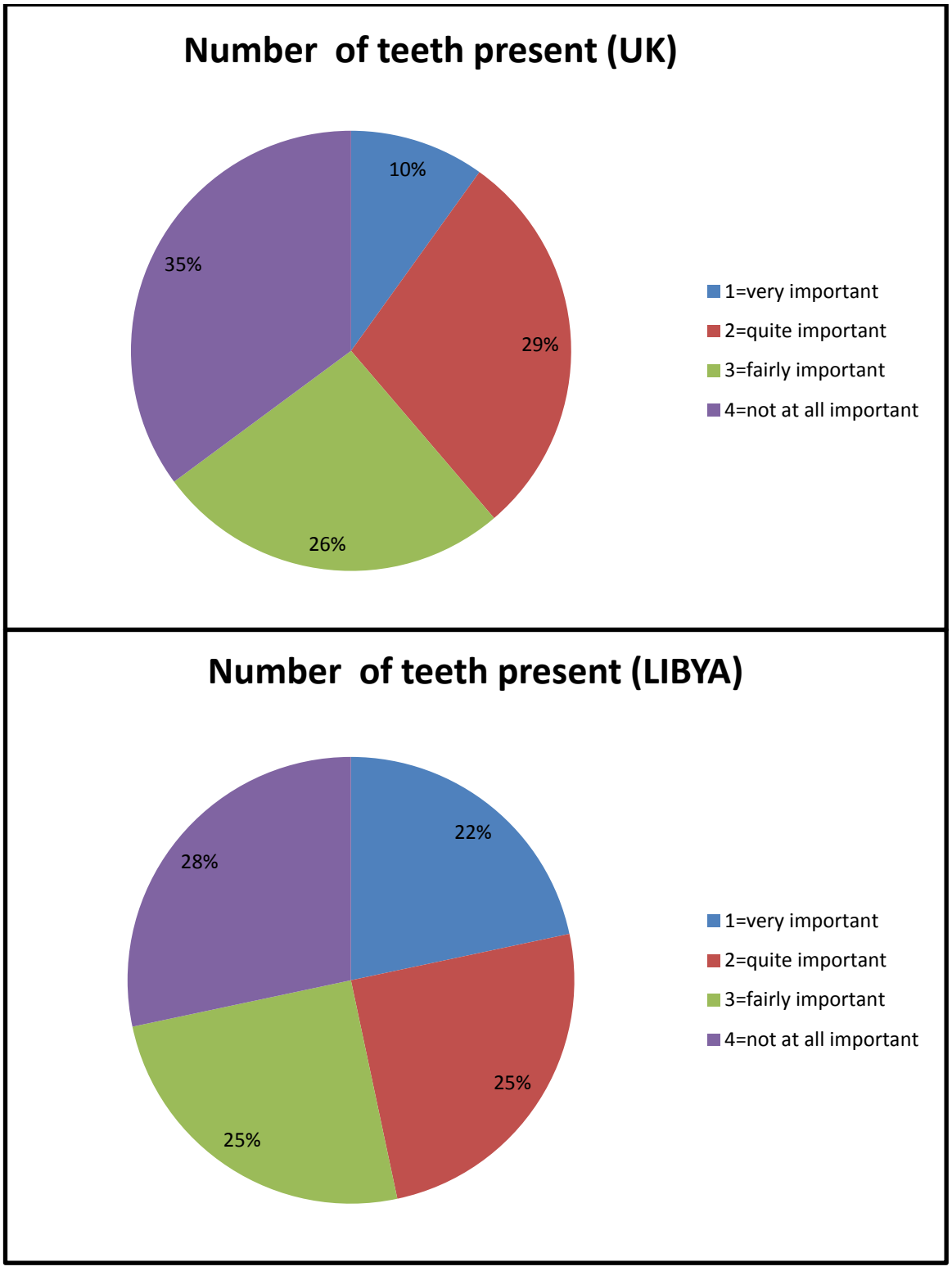


Figure 4-3 Number of teeth present.

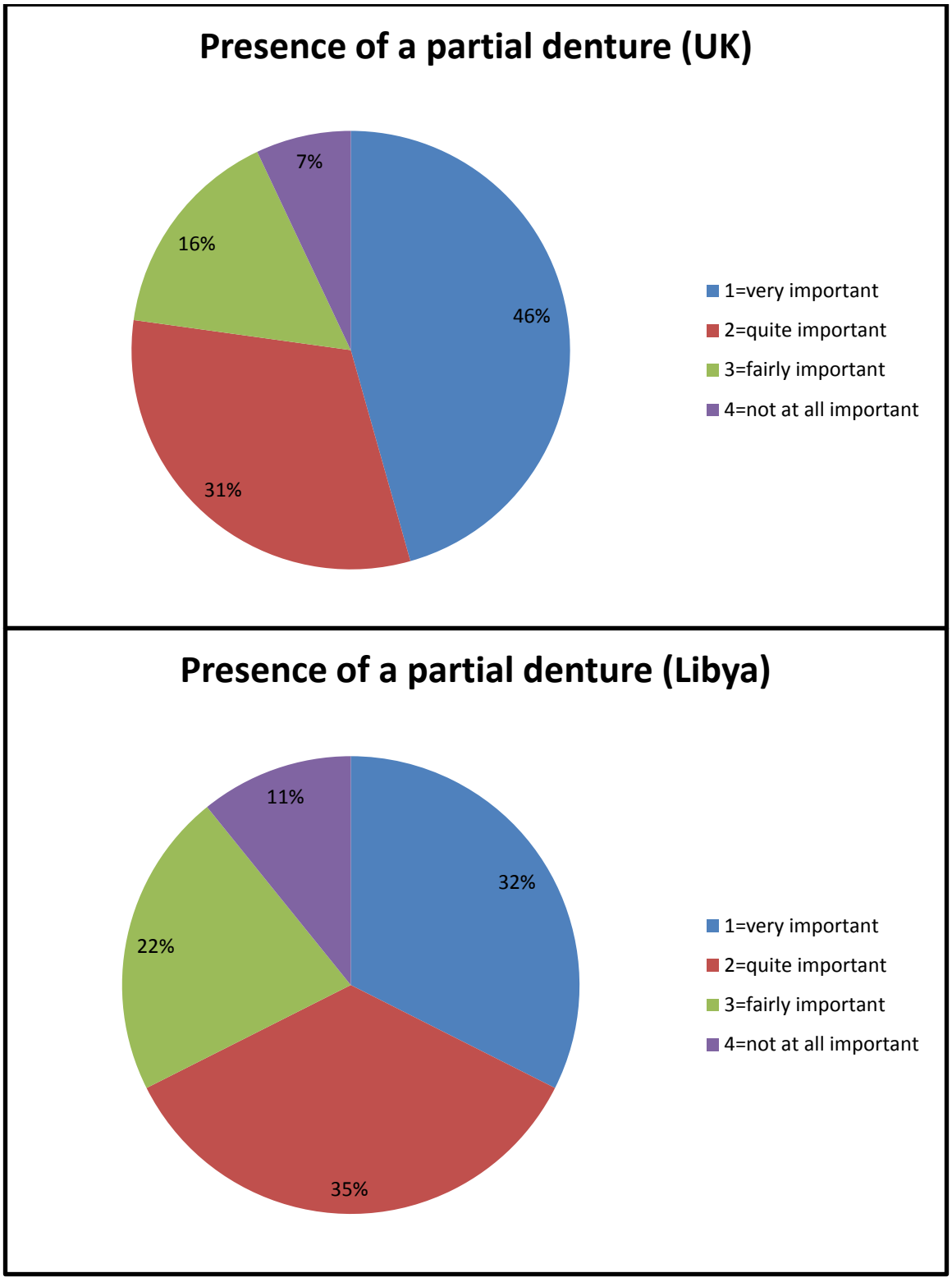


Figure 4-4 Presence of a partial denture.

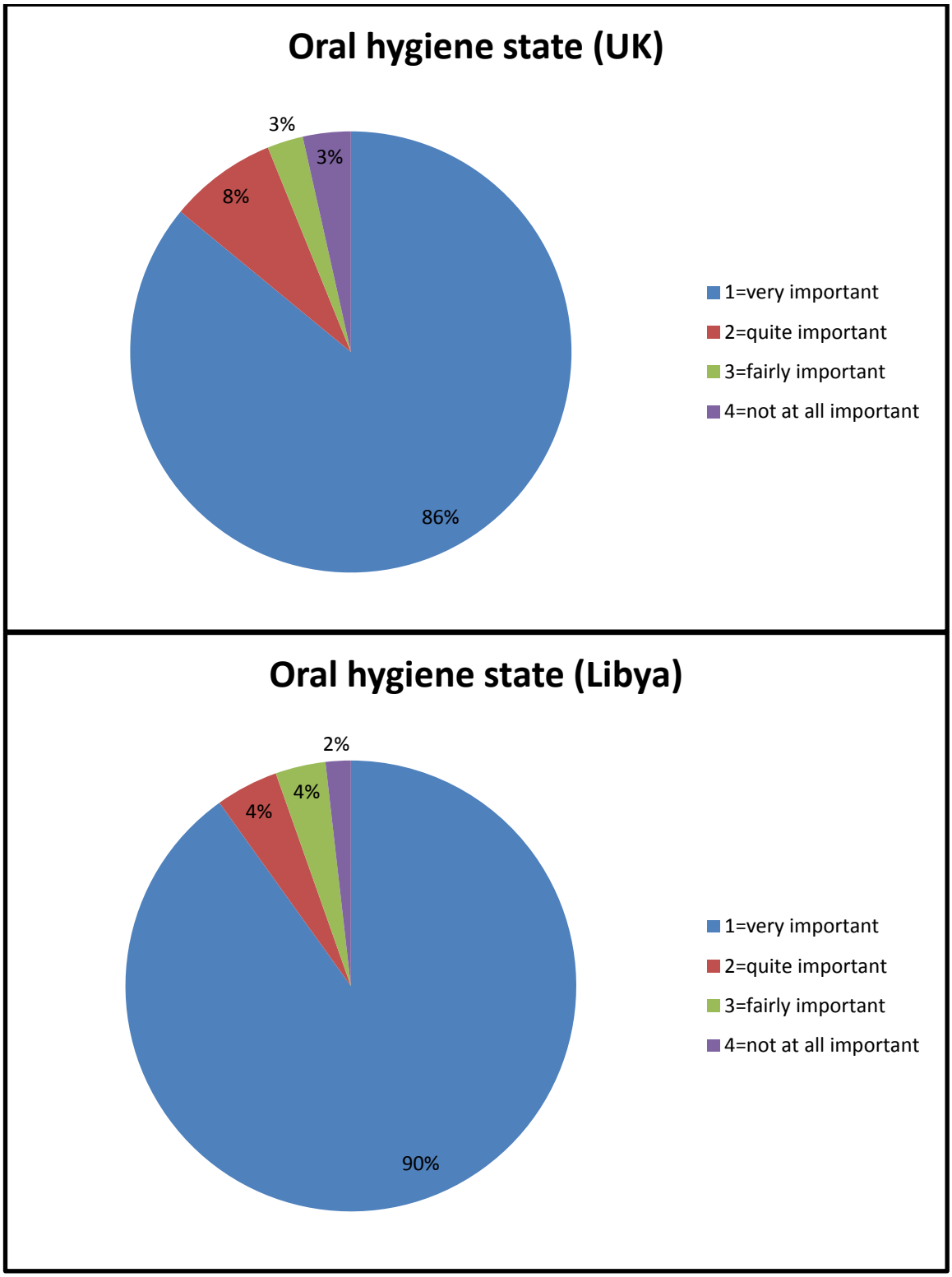


Figure 4-5 Oral hygiene states.

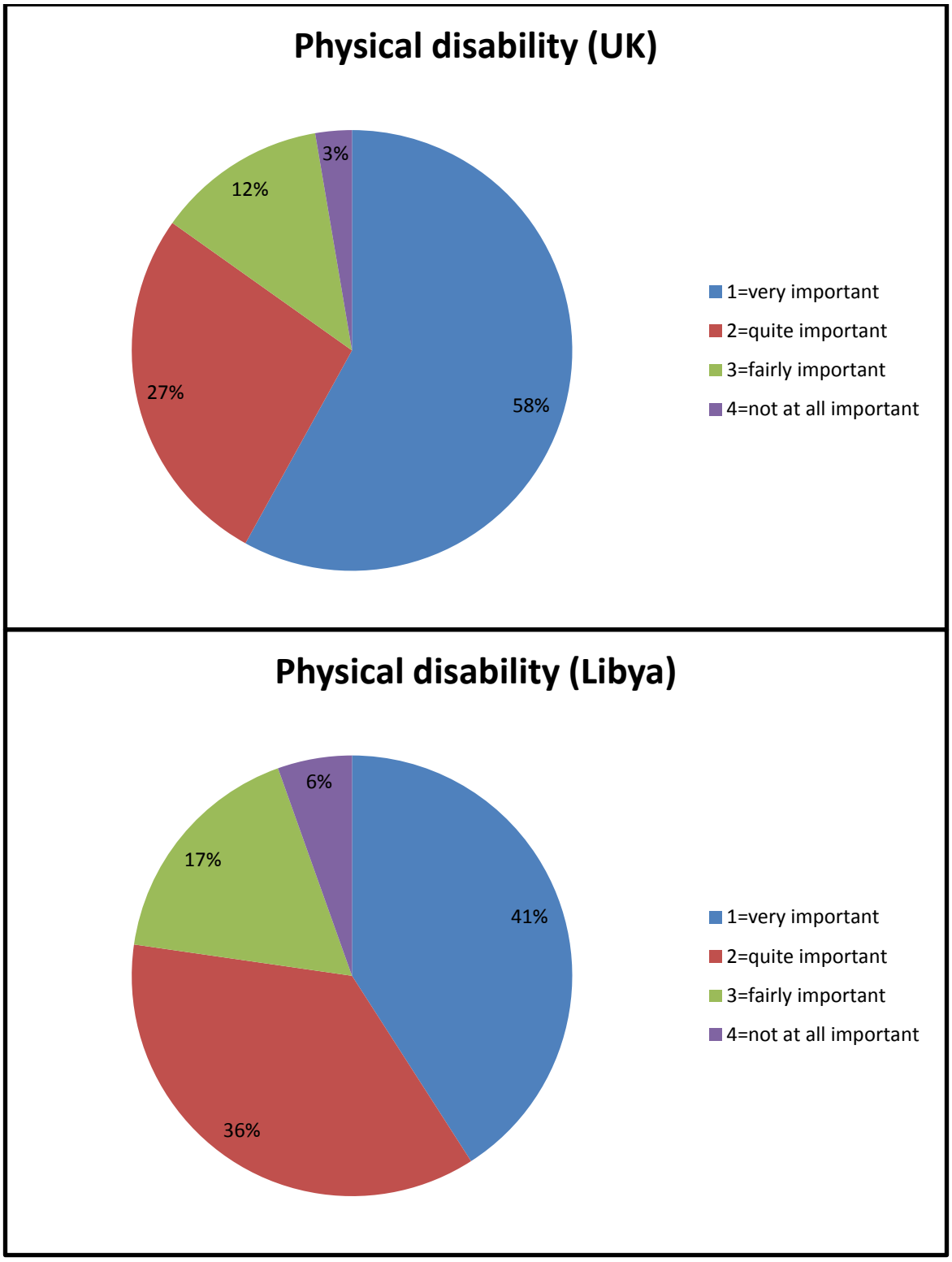


Figure 4-6 Physical disability.

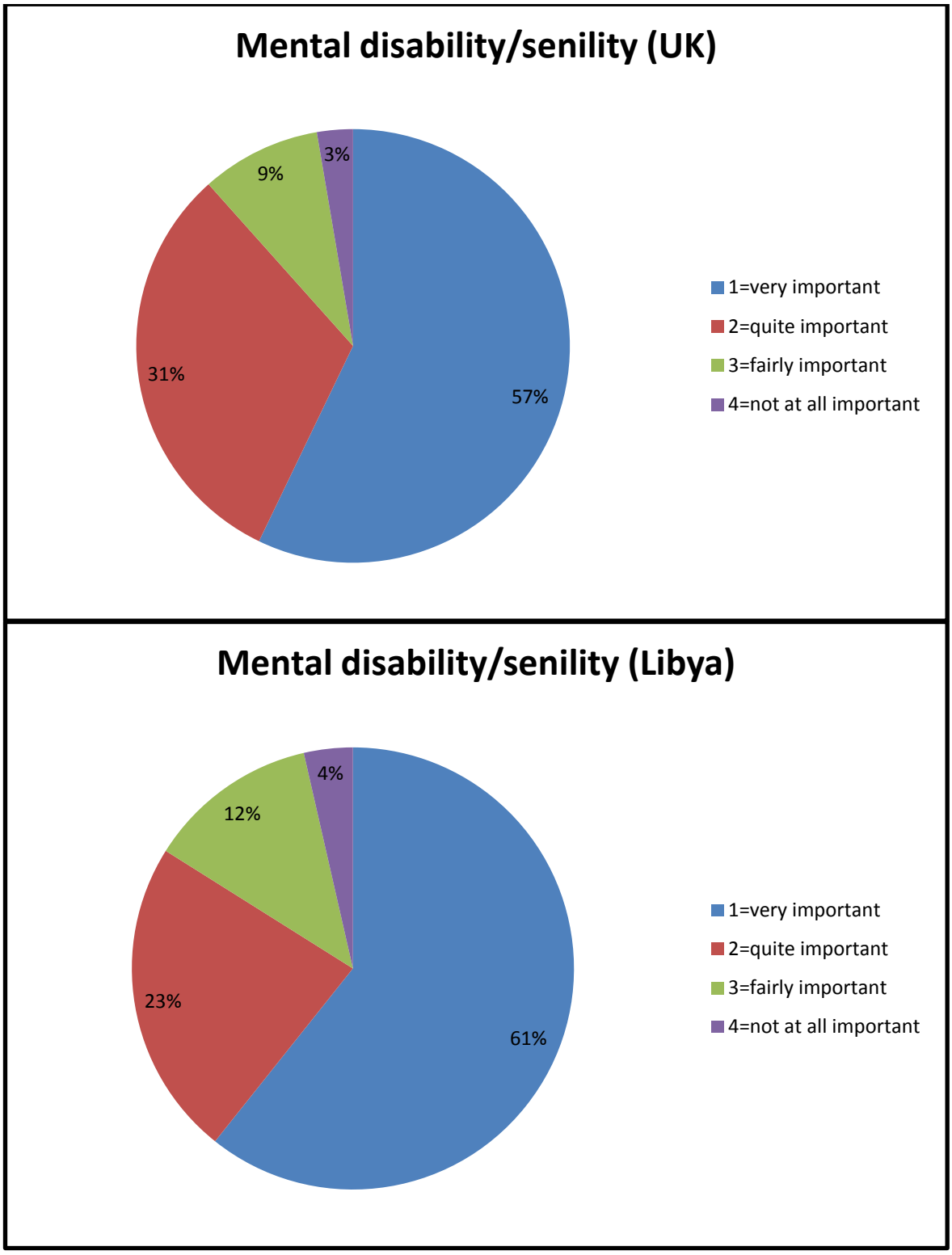


Figure 4-7 Mental disability/senility.

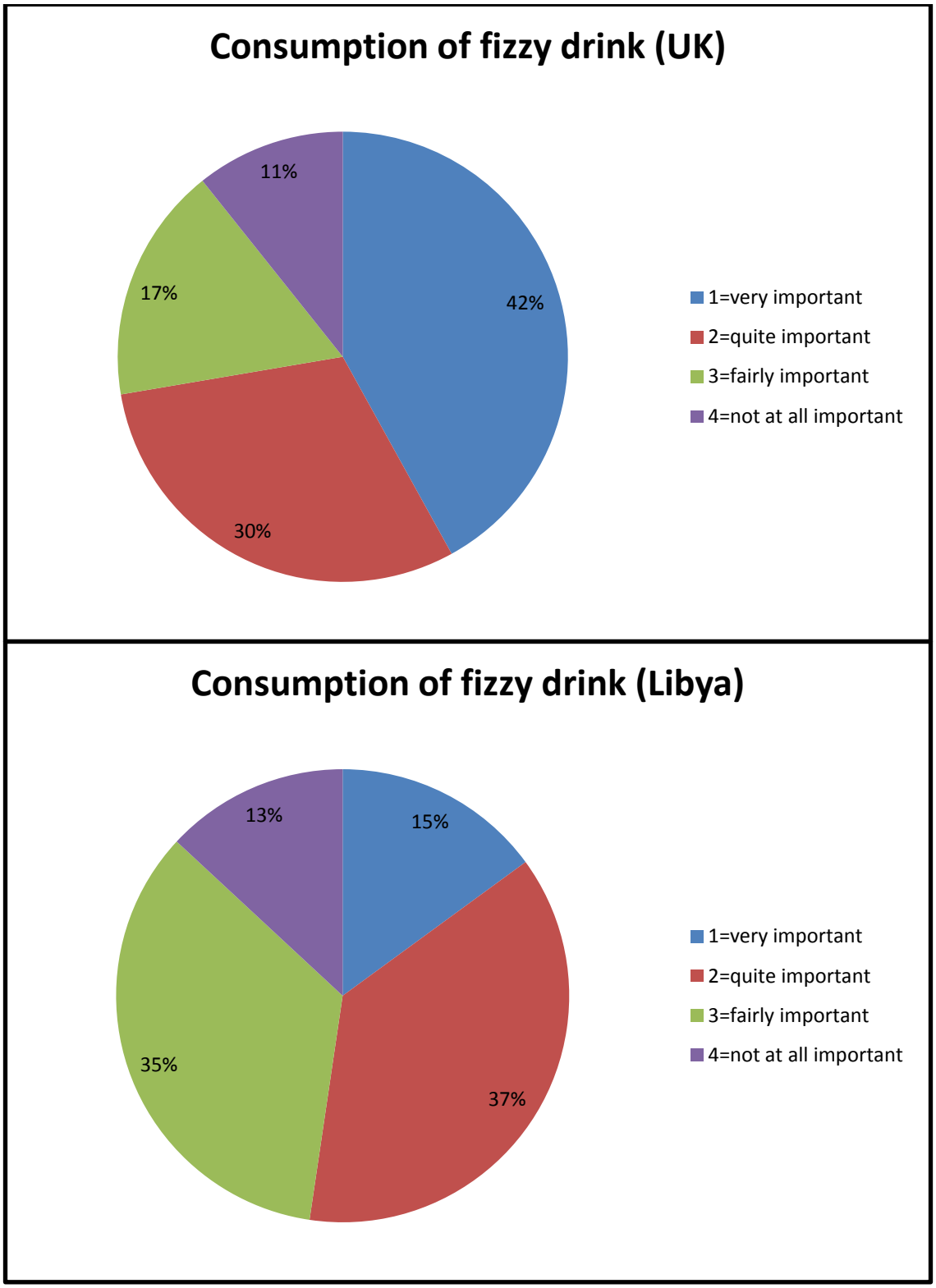


Figure 4-8 Consumption of fizzy drink.

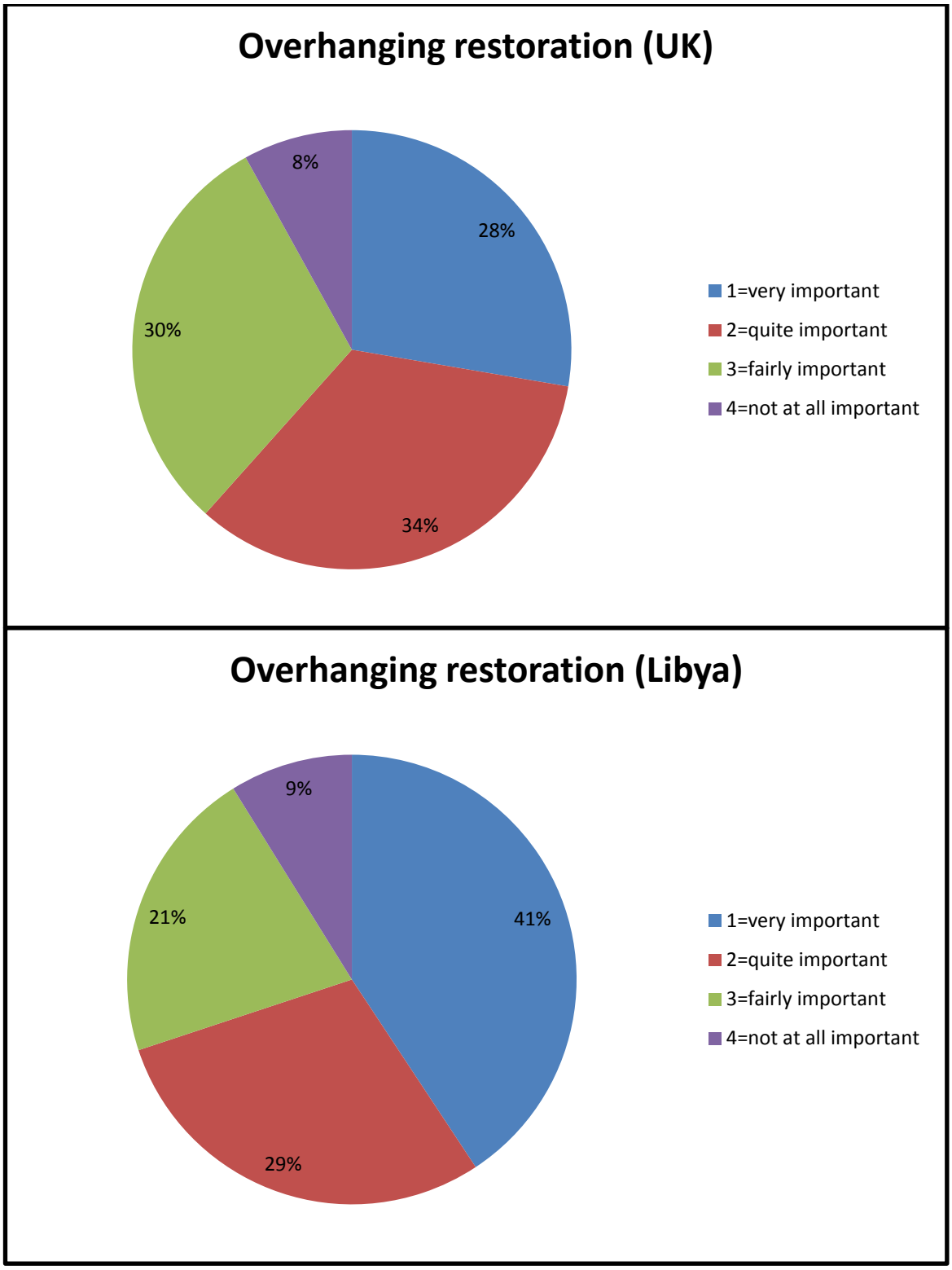


Figure 4-9 Overhanging restoration.

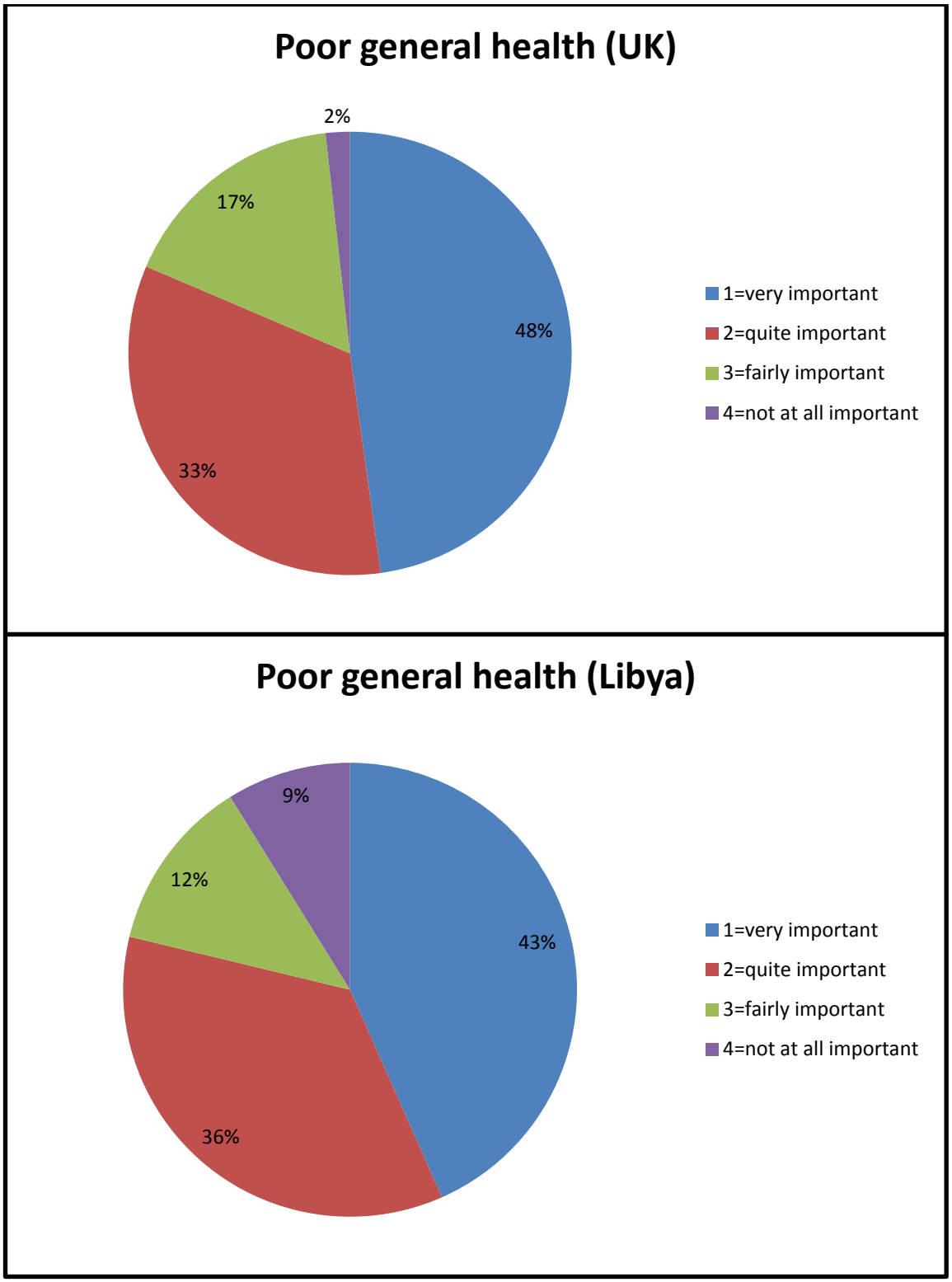


Figure 4-10 Poor general health.

Degree of crowding considered proportionally more important in Libya (figure 4-11)

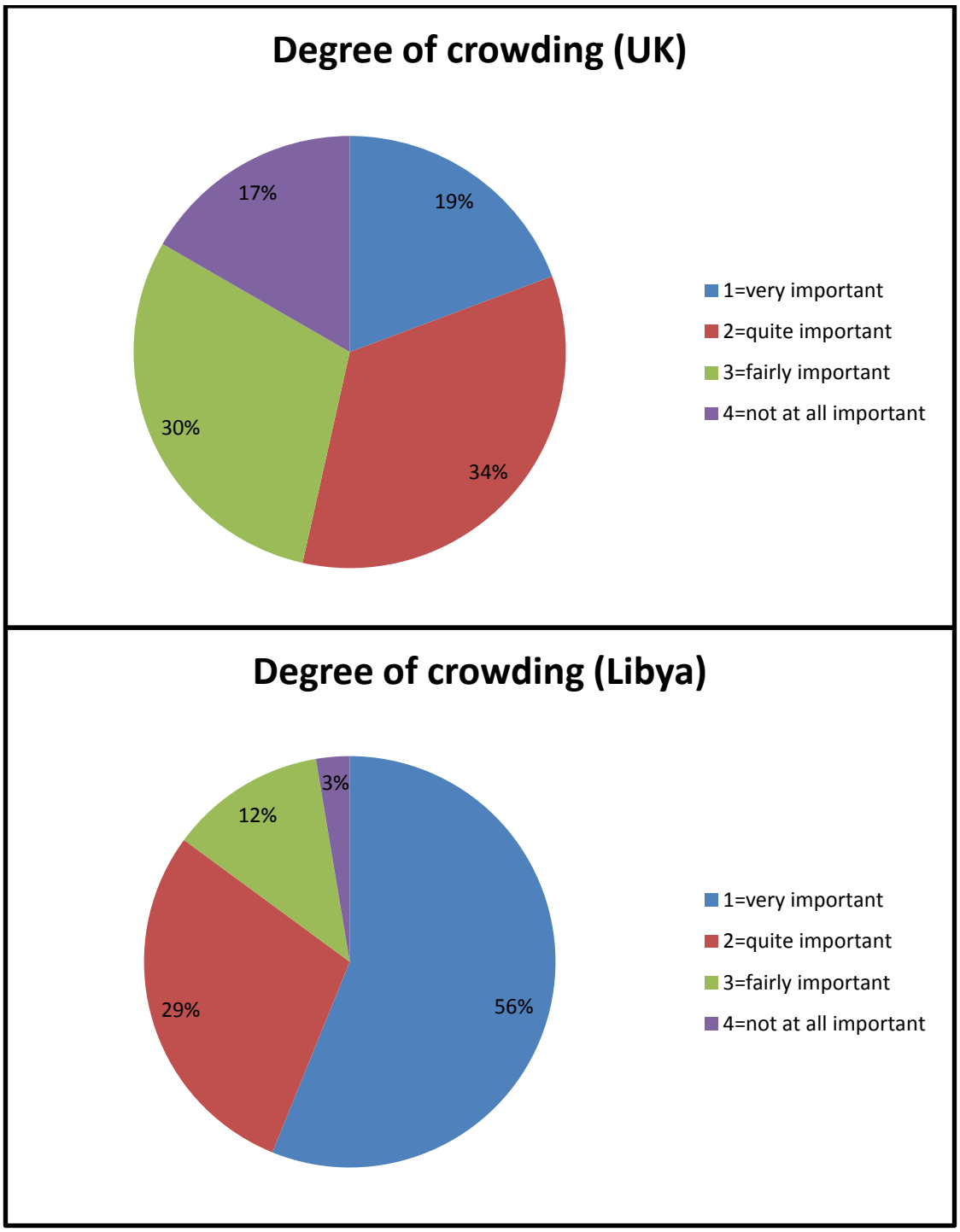


Figure 4-11 Degree of crowding.

Cigarette smoking considered proportionally more important in Libya (figure 4-12)

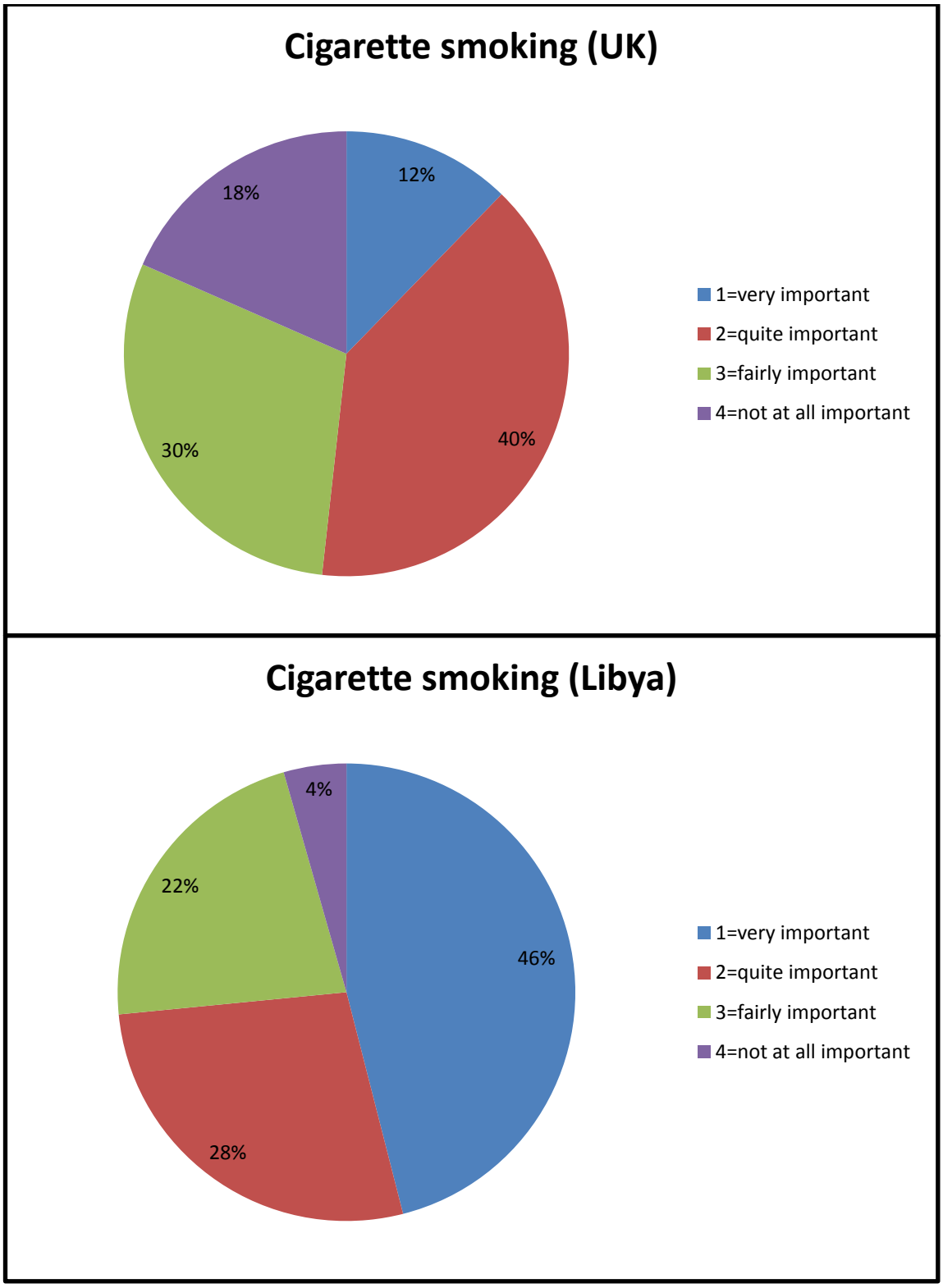


Figure 4-12 Cigarette smoking.

Total amount of sugars consumed considered proportionally more important in the UK (figure 4-13)

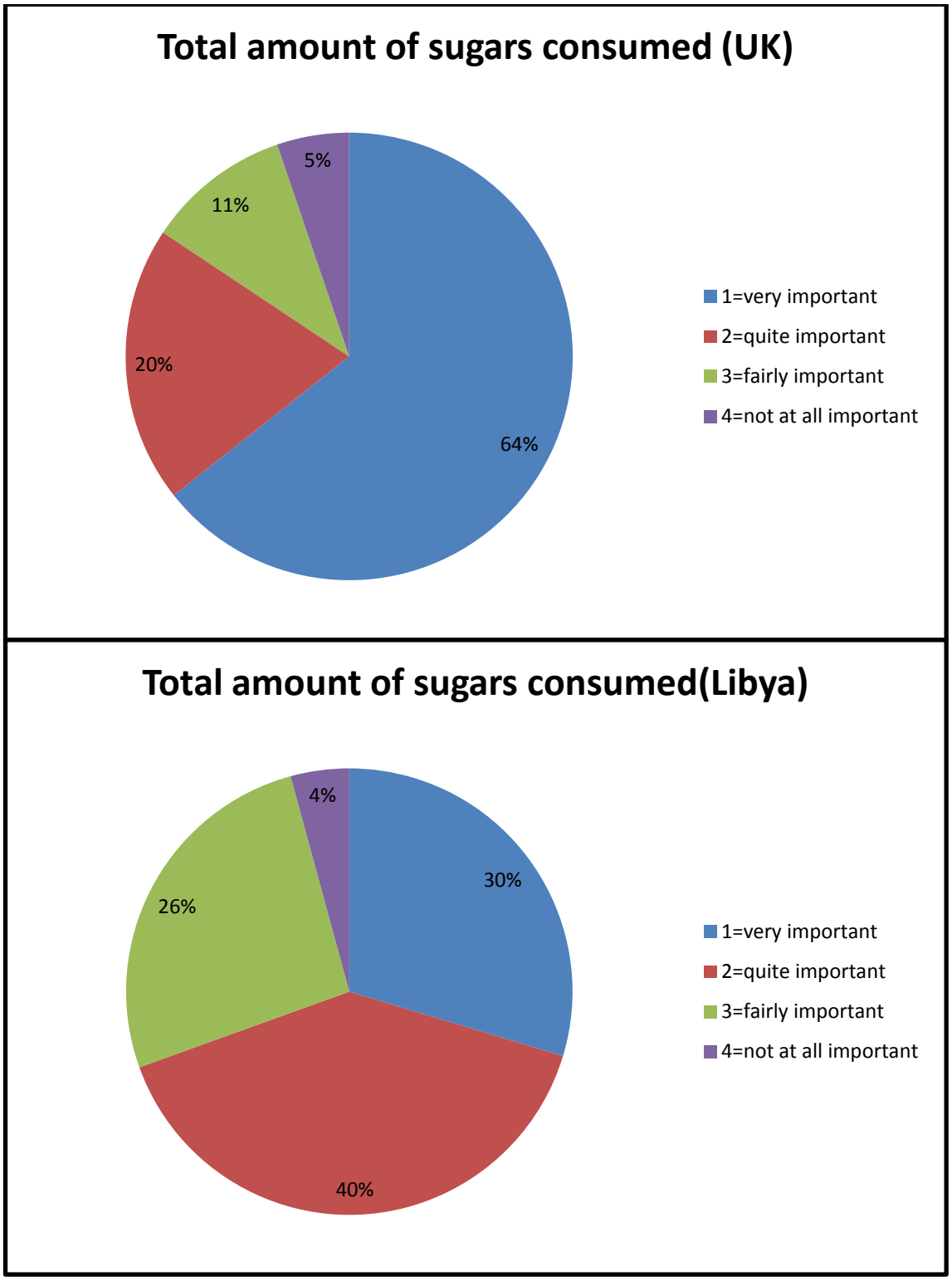


Figure 4-13 Total amount of sugars consumed.

Frequency of sugar intake considered proportionally more important in the UK (figure 4-14)

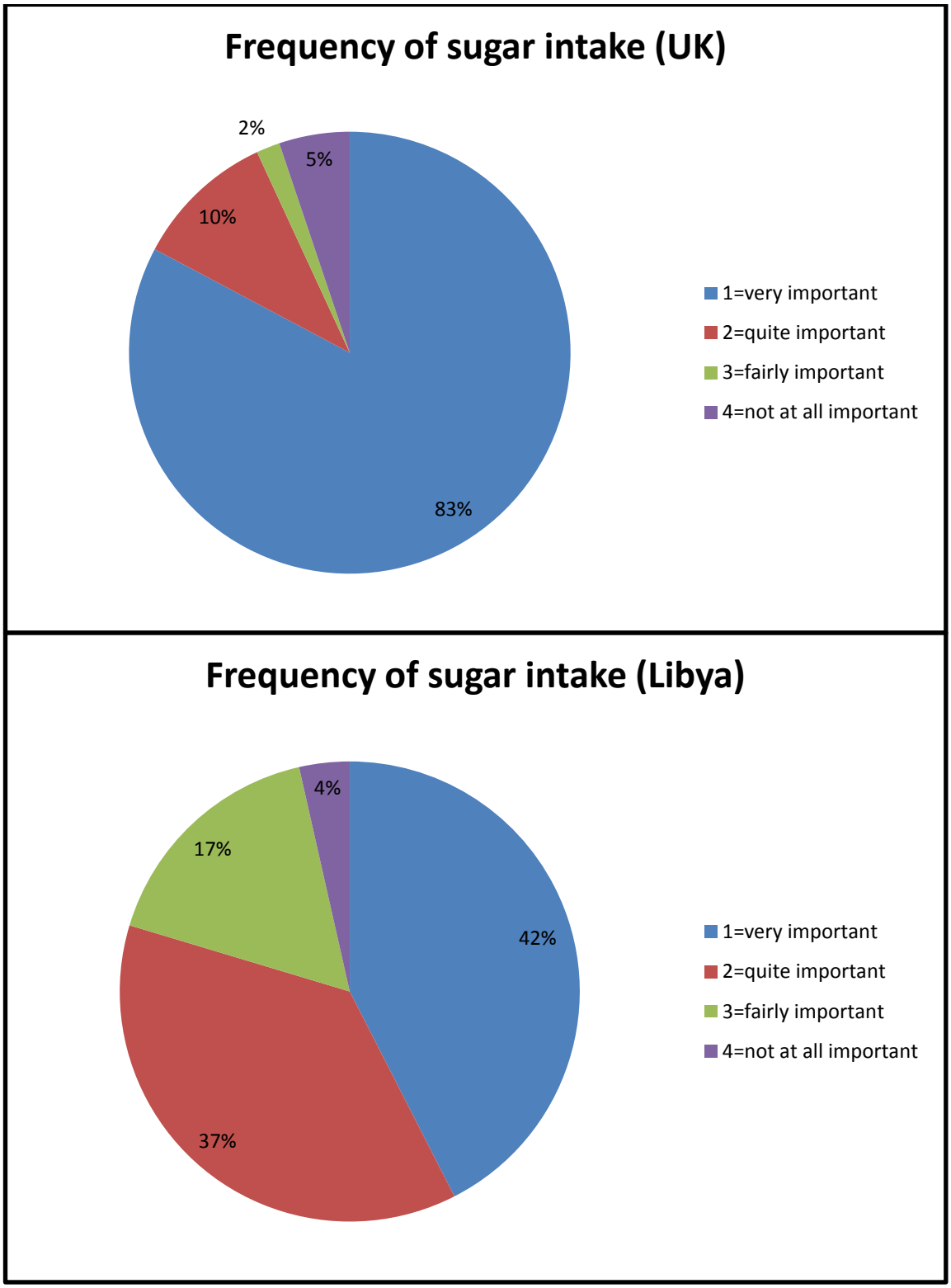


Figure 4-14 Frequency of sugar intake.

Active periodontal disease considered proportionally more important in Libya (figure 4-15)

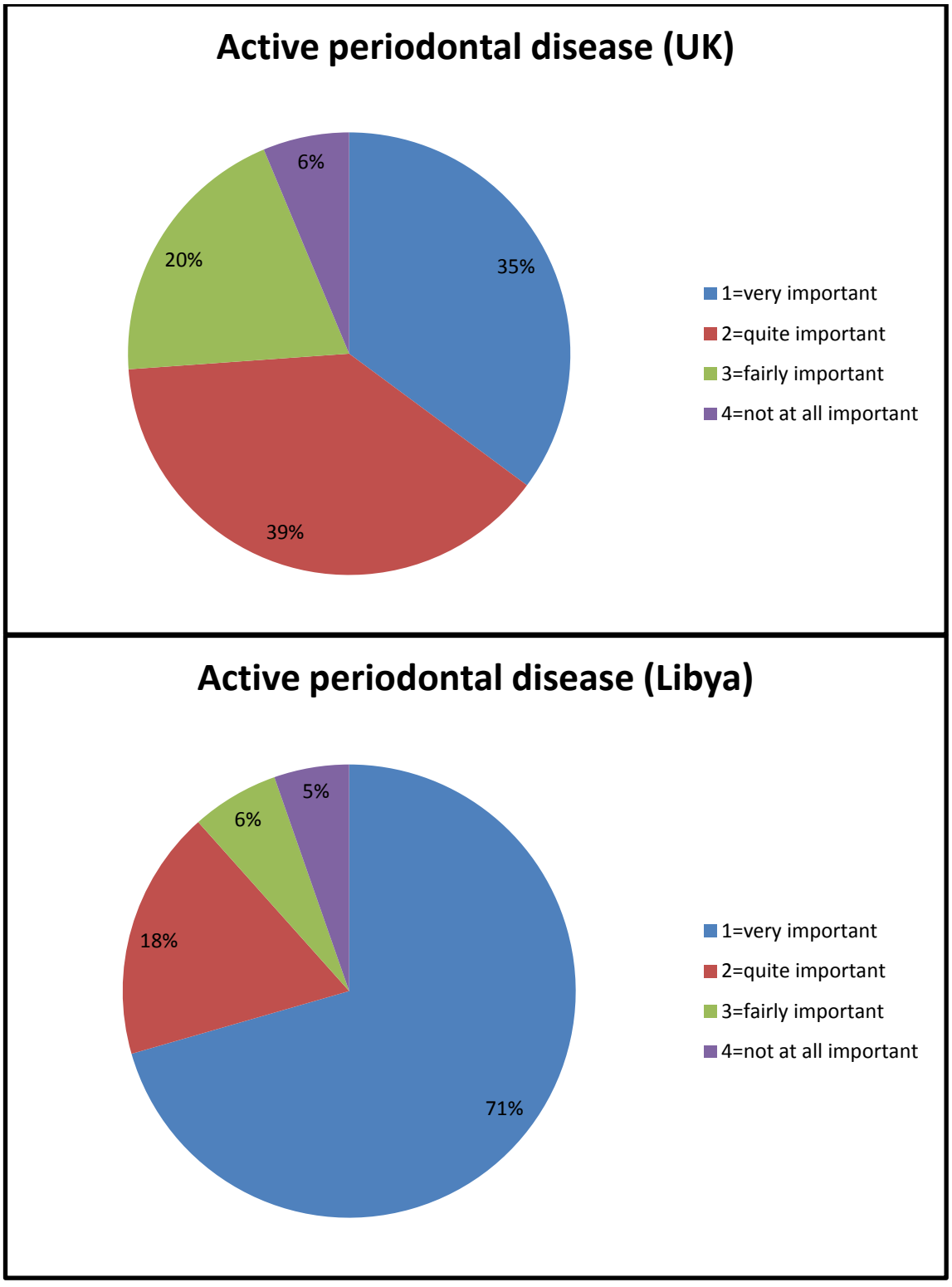


Figure 4-15 Active periodontal diseases.

Consumption of alcohol considered proportionally more important in Libya (figure 4-16)

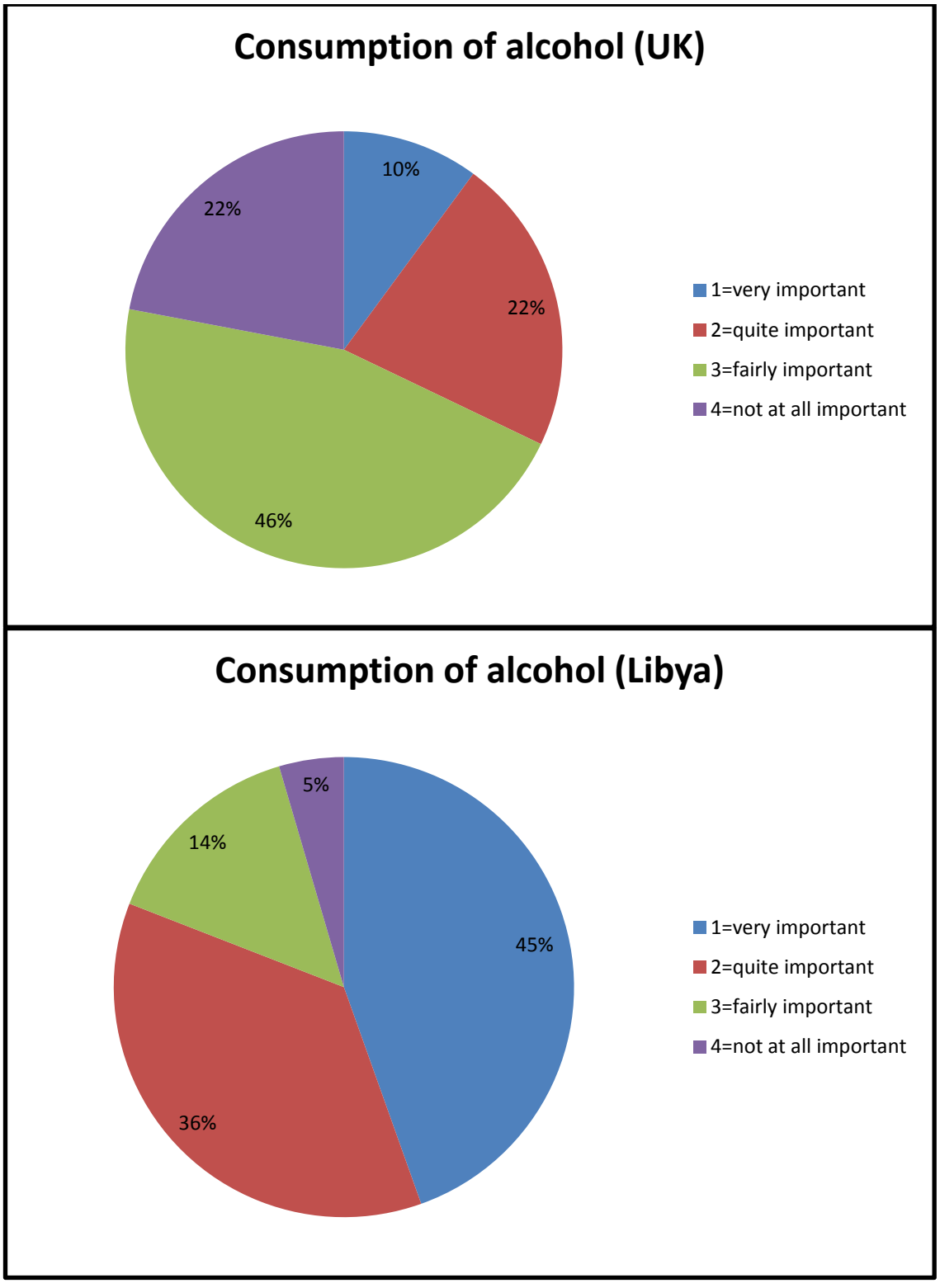


Figure 4-16 Consumption of alcohol.

Poor crown margins considered proportionally more important in Libya (figure 4-17)

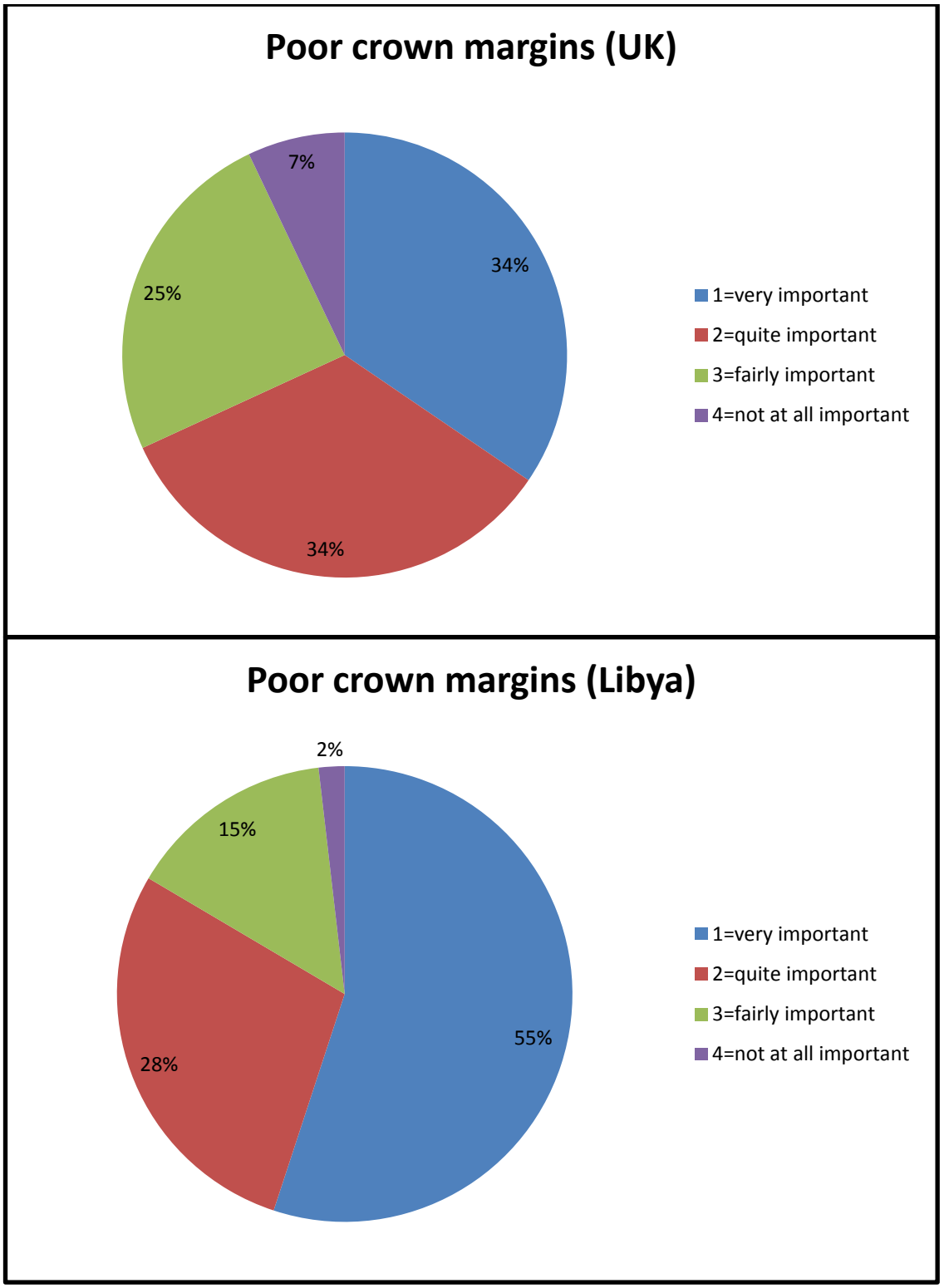


Figure 4-17 Poor crown margins.

Gingival recession considered proportional ly more important in Libya (figure 4-18)

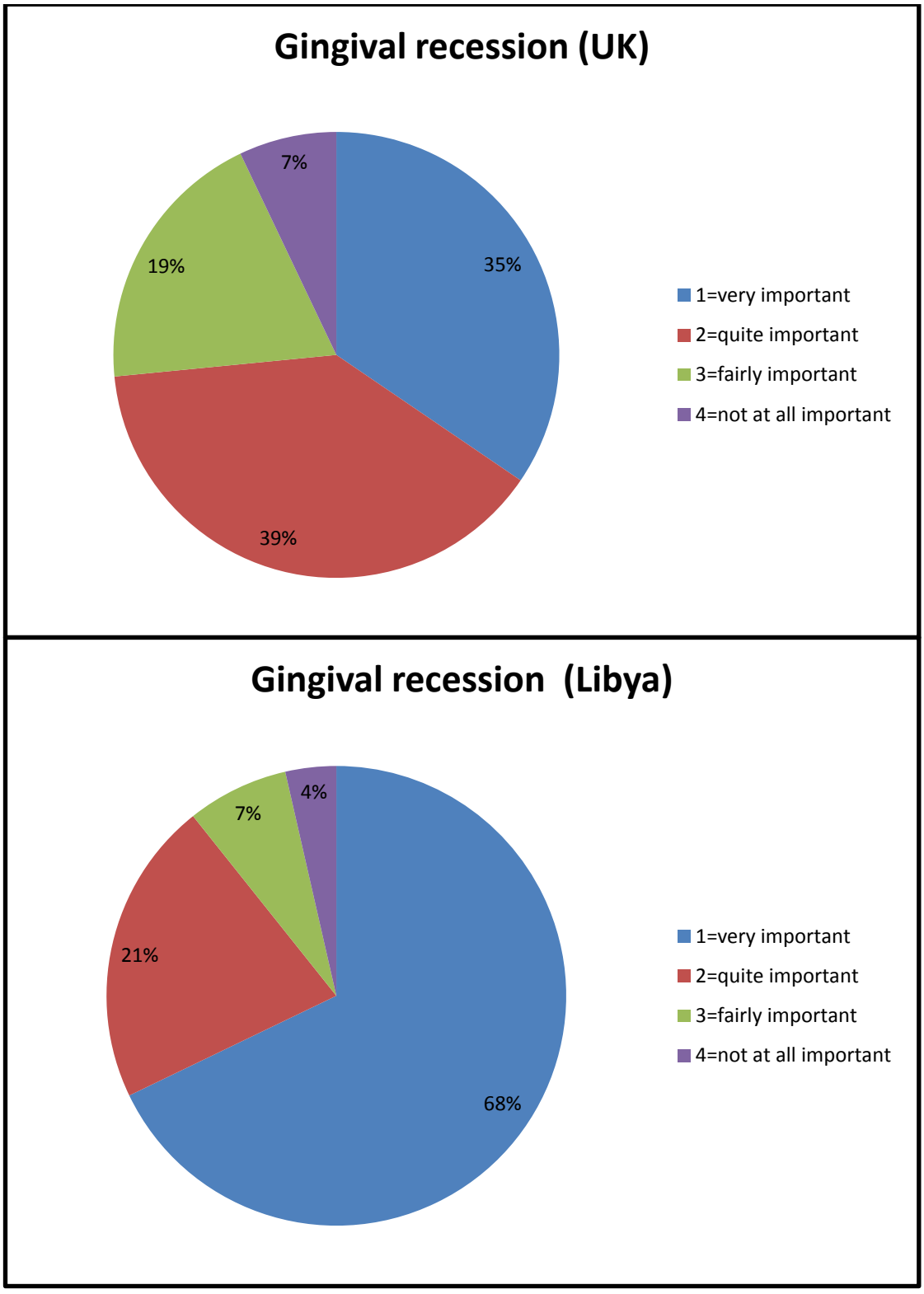


Figure 4-18 Gingival recession.

Reduce salivary flow considered proportionally more important in the UK (figure 4-19)

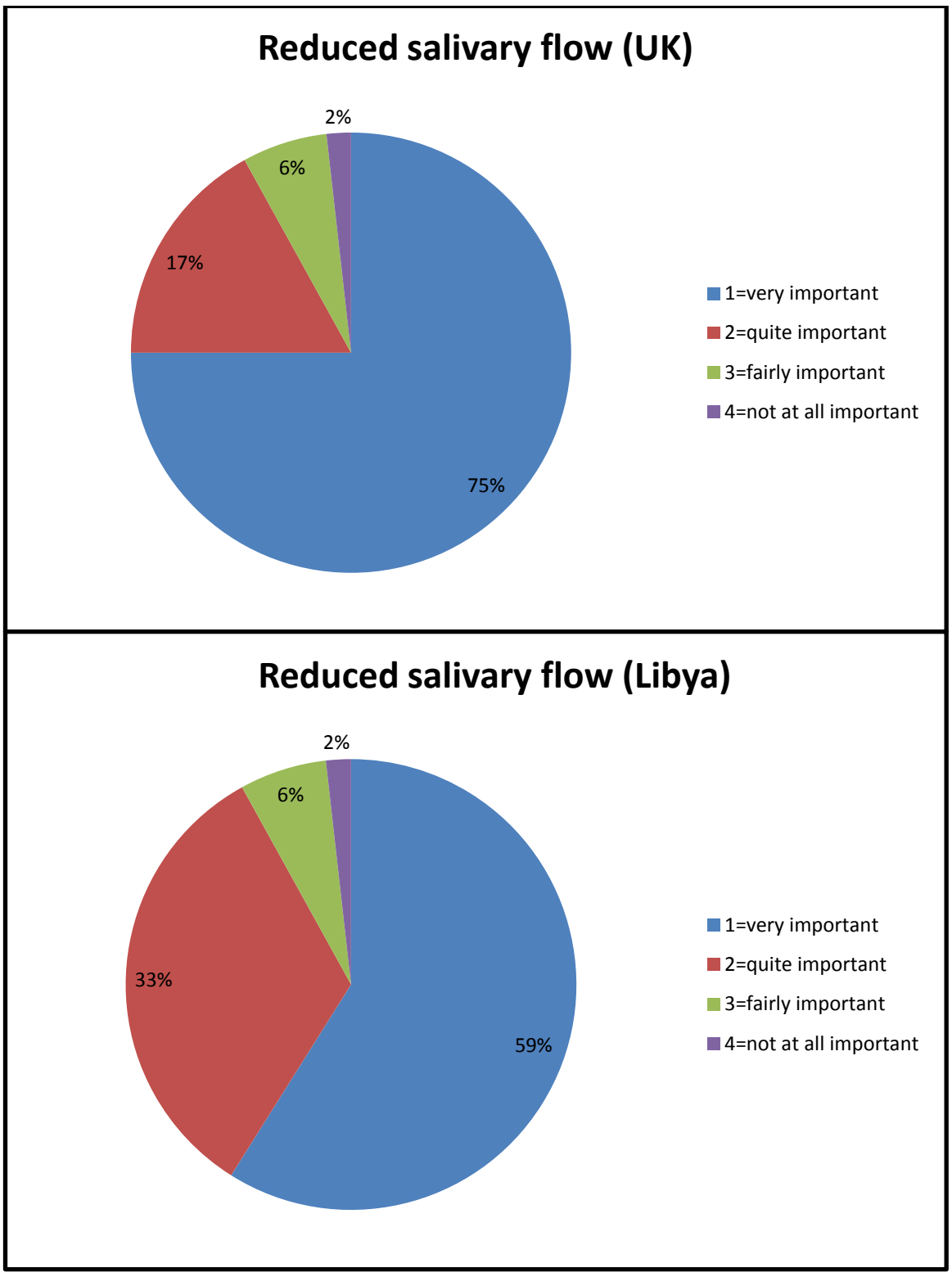


Figure 4-19 Reduce salivary flow.

Presence of erosion considered proportionally more important in Libya (figure 4-20)

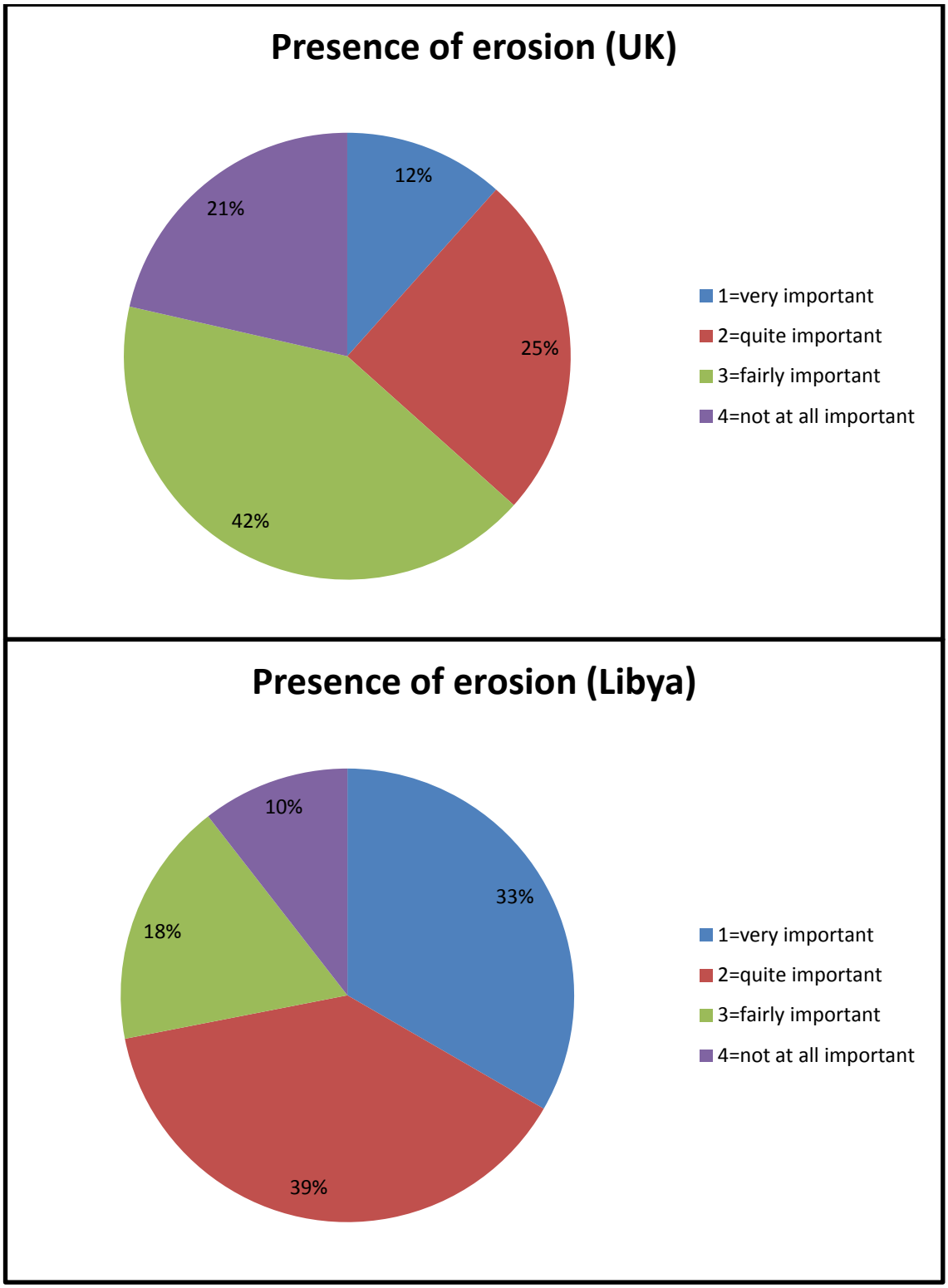


Figure 4-20 Presence of erosion.

Presence of abrasion cavity considered proportionally more important in Libya (figure 4-21)

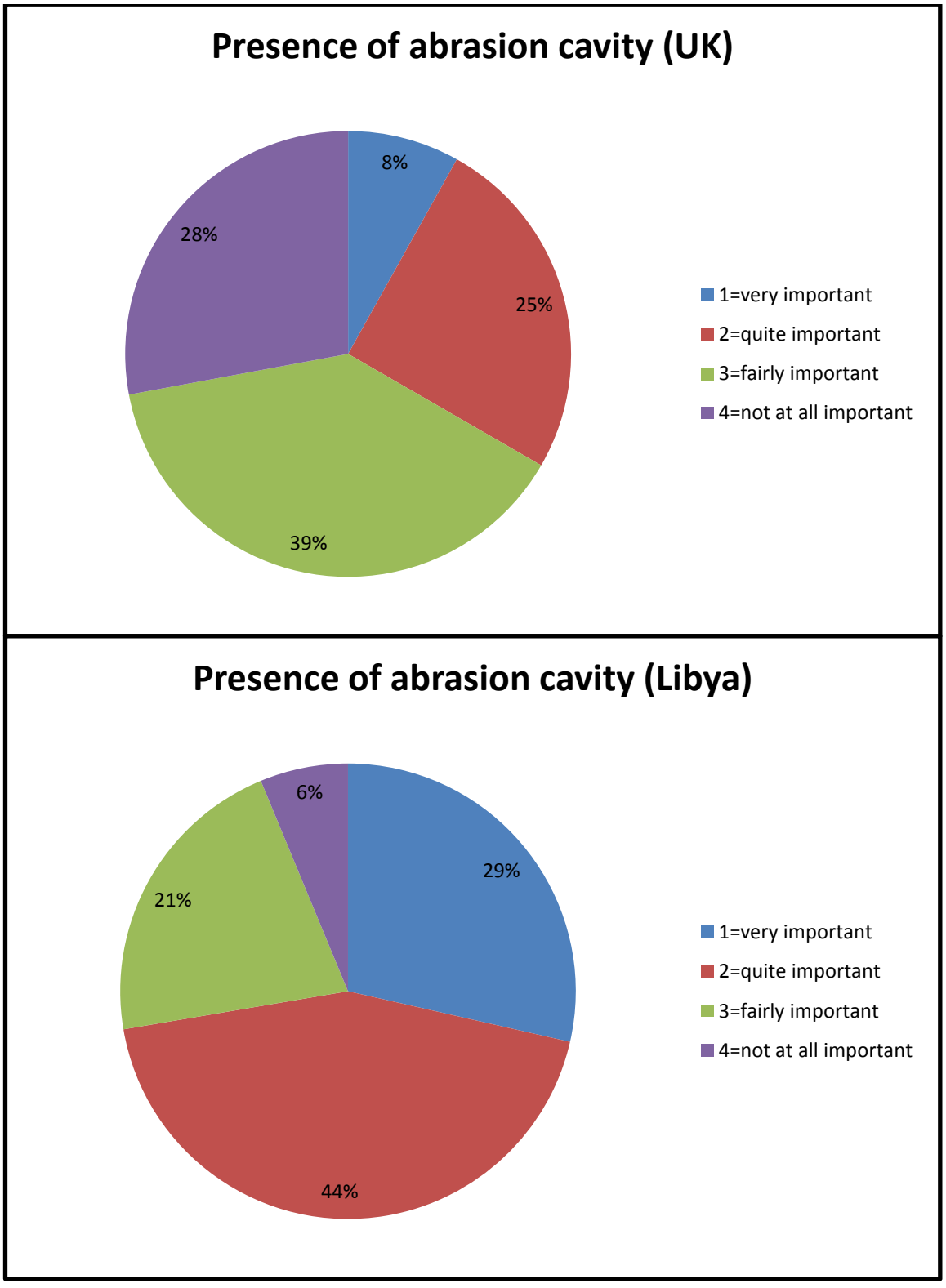


Figure 4-21 Presence of abrasion cavity.

4.2 THE LABORATORY INVESTIGATIONS

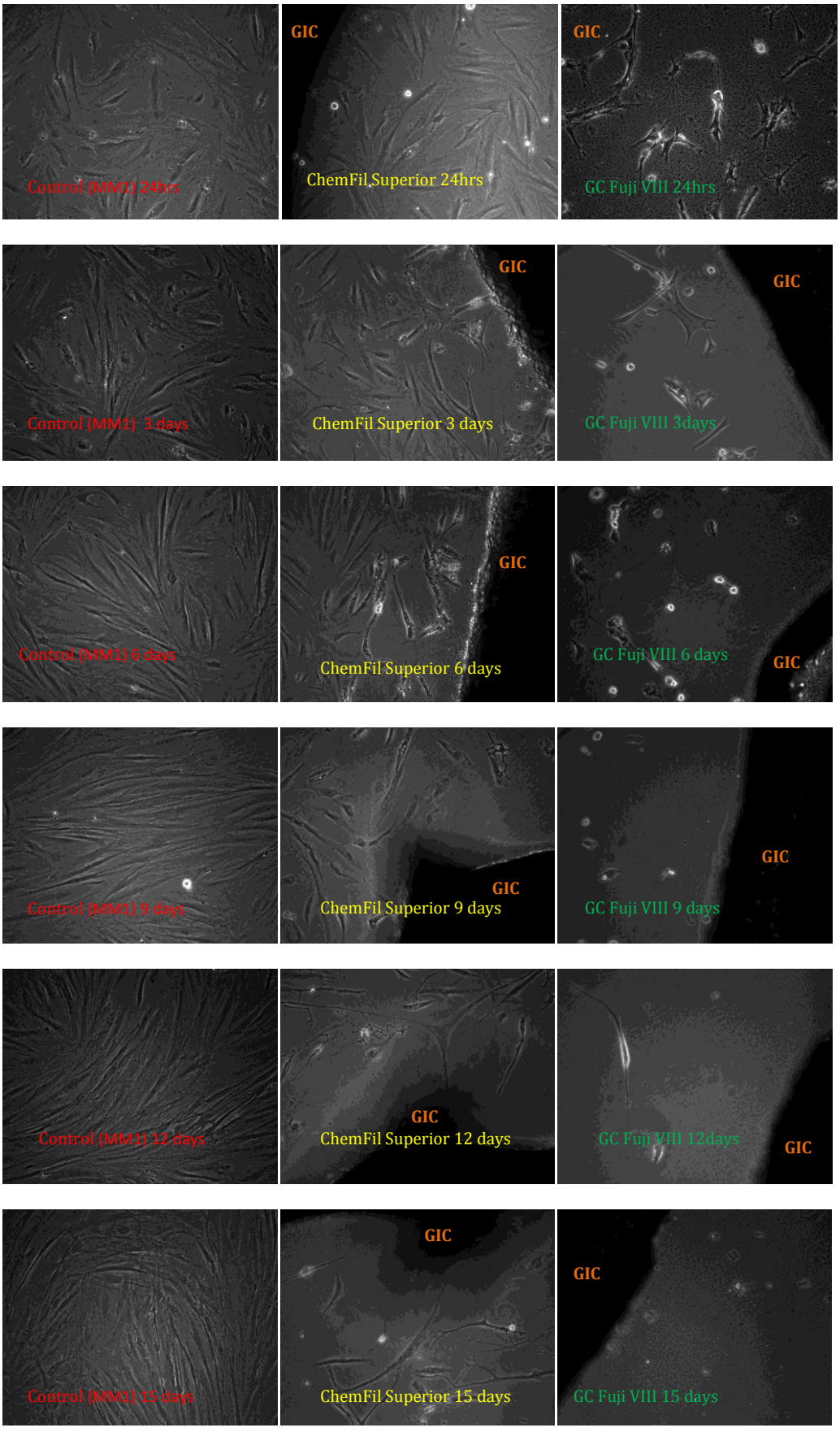
4.2.1THE INVESTIGATION OF BIOACTIVE ADDITIONS TO GLASS POLYALKENOATE CEMENTS

4.2.1.1 *OBSERVATION UNDER THE MICROSCOPE*

In order to evaluate the visible effect of materials on oral mucosa cells the cells were cultured along with materials specimens for 21days. During this period the cells were observed and monitored under a light microscope and pictures were taken every 3 days figures 4-22 to 4-31. Such images were ranked by ten observers. The raw data obtained is contained in appendices D, E, F, G and, H

4.2.1.1.1 DETERMINATION OF THE OPTIMAL MATERIALS FOR ATTACHMENT OF CELLES.

The oral mucosa fibroblast cells cultured around ChemFil superior and GC Fuji VIII for 21 days. The pictures were taken every three days close and away to the specimens Figures 4-22- 4-23.


Control (MM1) 24hrs
GIC
ChemFil Superior 24hrs
GIC
GC Fuji VIII 24hrs
Control (MM1) 3 days
GIC
ChemFil Superior 3 days
GIC
GC Fuji VIII 3days
Control (MM1) 6 days
GIC
ChemFil Superior 6 days
GC Fuji VIII 6 days
GIC
Control (MM1) 9 days
GIC
ChemFil Superior 9 days
GIC
GC Fuji VIII 9 days
Control (MM1) 12 days
GIC
ChemFil Superior 12 days
GC Fuji VIII 12days
GIC
Control (MM1) 15 days
GIC
ChemFil Superior 15 days
GIC
GC Fuji VIII 15 days

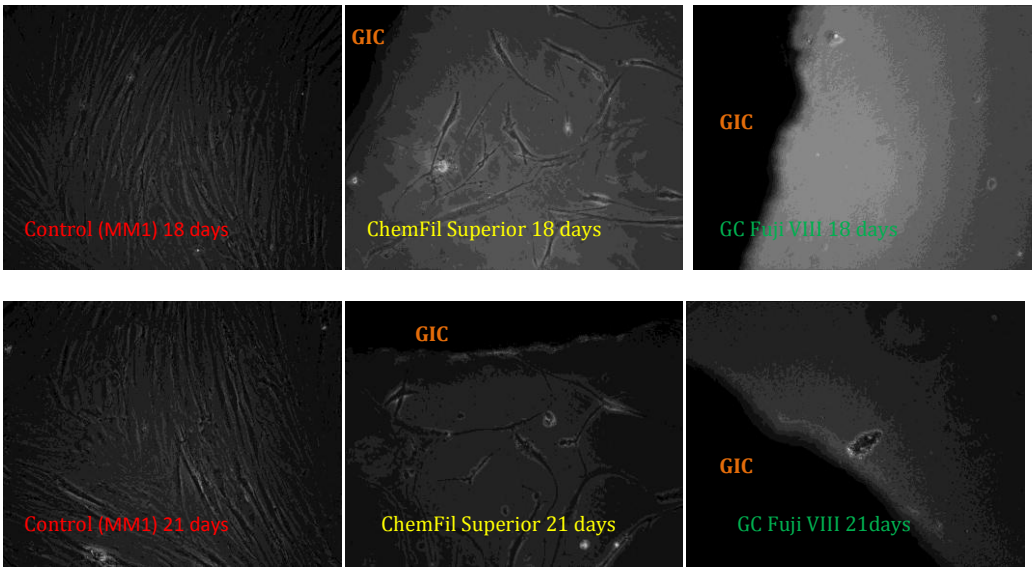
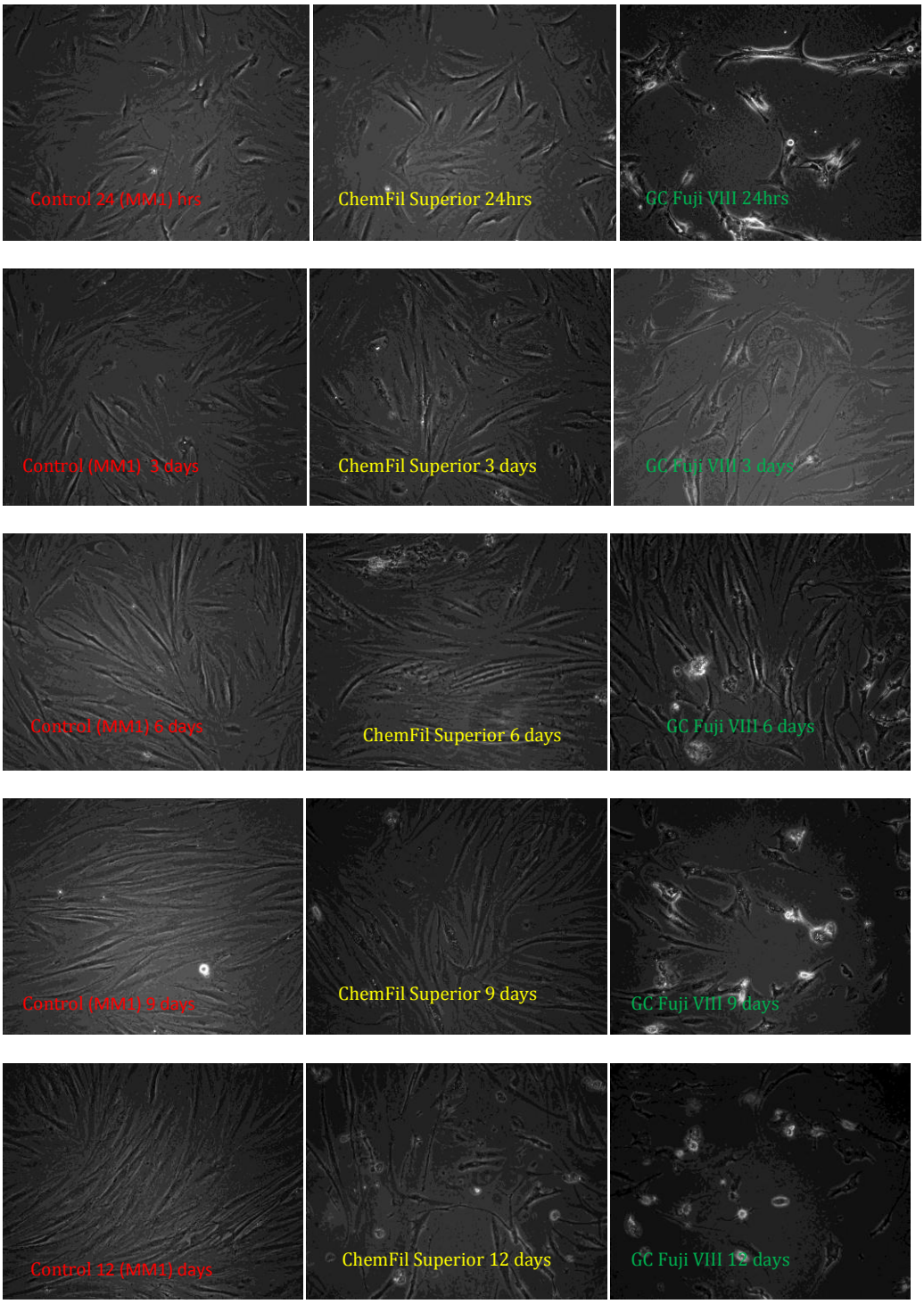


Figure 4-22 The oral mucosa fibroblast cells cultured close to ChemFil superior and GC Fuji VIII specimens for 21 days.


Control 24 (MM1) hrs
ChemFil Superior 24hrs
GC Fuji VIII 24hrs
Control (MM1) 3 days
ChemFil Superior 3 days
GC Fuji VIII 3 days
Control (MM1) 6 days
ChemFil Superior 6 days
GC Fuji VIII 6 days
Control (MM1) 9 days
ChemFil Superior 9 days
GC Fuji VIII 9 days
Control 12 (MM1) days
ChemFil Superior 12 days
GC Fuji VIII 12 days

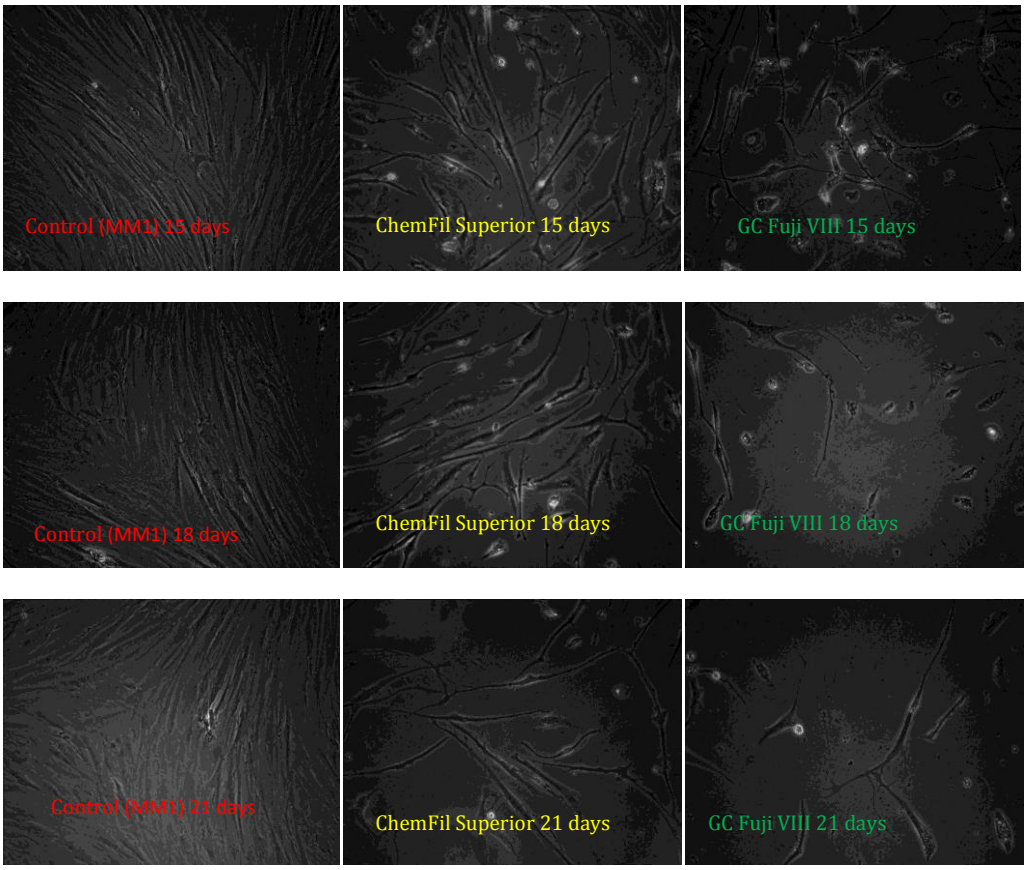


Figure 4-23 The oral mucosa fibroblast cells cultured away from ChemFil superior and GC Fuji VIII specimens for 21 days.

A Kruskal-Wallis test demonstrated that, very highly statistically significant differences ($P < 0.0001$) between the groups of the pooled rankings of cell viabilities of the oral mucosal cells located in contact and away from the materials seen over all observation times. Localisation by a Dunn's Multiple comparison test showed that all groups different significantly ($P < 0.001$) from each other. The most common ranking scores for each group were,

- Control = Rank 1 (Range = 2, Min Rank = 1, Max Rank = 3).
- ChemFil Superior (Close) = Rank 3 (Range = 3, Min Rank = 1, Max Rank = 4)
- GC Fuji VIII (Close) = Rank 4 (Range = 2, Min Rank =3, Max Rank = 4)

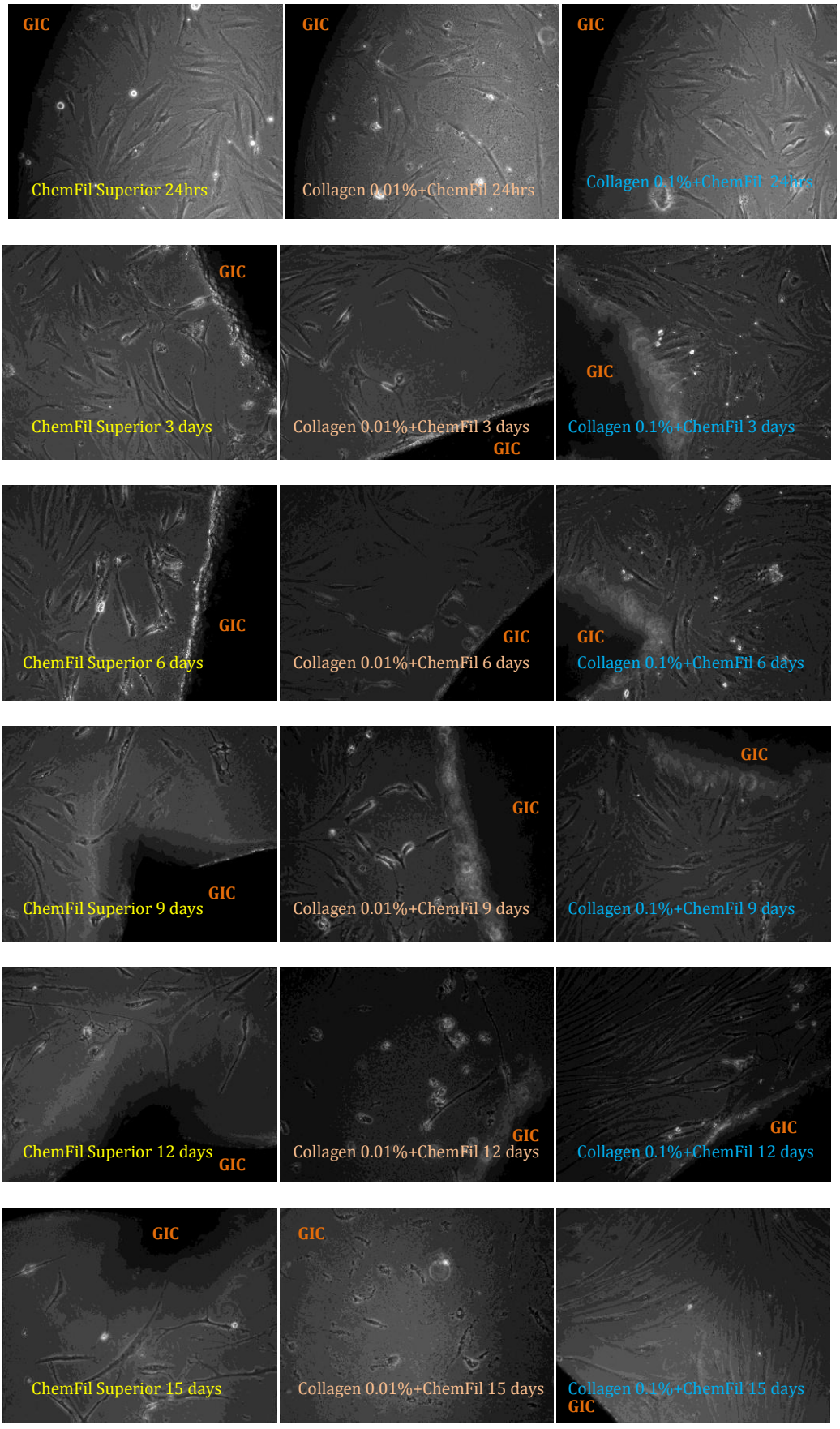
- Control (Away) = Rank 1 (Range = 1, Min Rank = 1, Max Rank = 2).
- ChemFil Superior (Away) = Rank 2 (Range = 2, Min Rank = 2, Max Rank = 4)
- GC Fuji VIII (Away) = Rank 4 (Range = 2, Min Rank =2, Max Rank = 4)

It is thus evident that the lowest cell viability was associated with GC Fuji VIII.

4.2.1.1.2 DETERMINATION OF THE OPTIMAL BIOACTIVE ADDITIVES AND CONCENTRATION

- **TYPE I COLLAGEN OPTIMAL CONCENTRATION**

The oral mucosa fibroblast cells cultured around unmodified and bio-modified ChemFil superior with different concentration of Type I collagen for 21 days. The pictures to the cells were taken every 3 days close and away to the specimens Figure 4-24 and 4-25.


GIC
ChemFil Superior 24hrs
GIC
Collagen 0.01%+ChemFil 24hrs
GIC
Collagen 0.1%+ChemFil 24hrs
GIC
ChemFil Superior 3 days
Collagen 0.01%+ChemFil 3 days
GIC
GIC
Collagen 0.1%+ChemFil 3 days
GIC
ChemFil Superior 6 days
GIC
Collagen 0.01%+ChemFil 6 days
GIC
GIC
Collagen 0.1%+ChemFil 6 days
GIC
ChemFil Superior 9 days
GIC
Collagen 0.01%+ChemFil 9 days
GIC
Collagen 0.1%+ChemFil 9 days
ChemFil Superior 12 days
GIC
GIC
Collagen 0.01%+ChemFil 12 days
GIC
Collagen 0.1%+ChemFil 12 days
GIC
ChemFil Superior 15 days
GIC
Collagen 0.01%+ChemFil 15 days
Collagen 0.1%+ChemFil 15 days
GIC

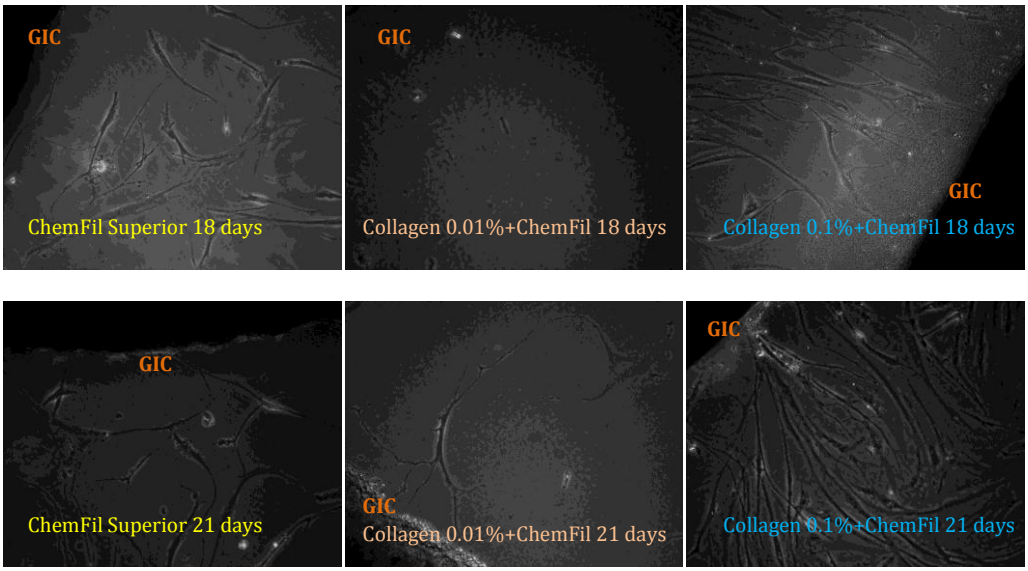
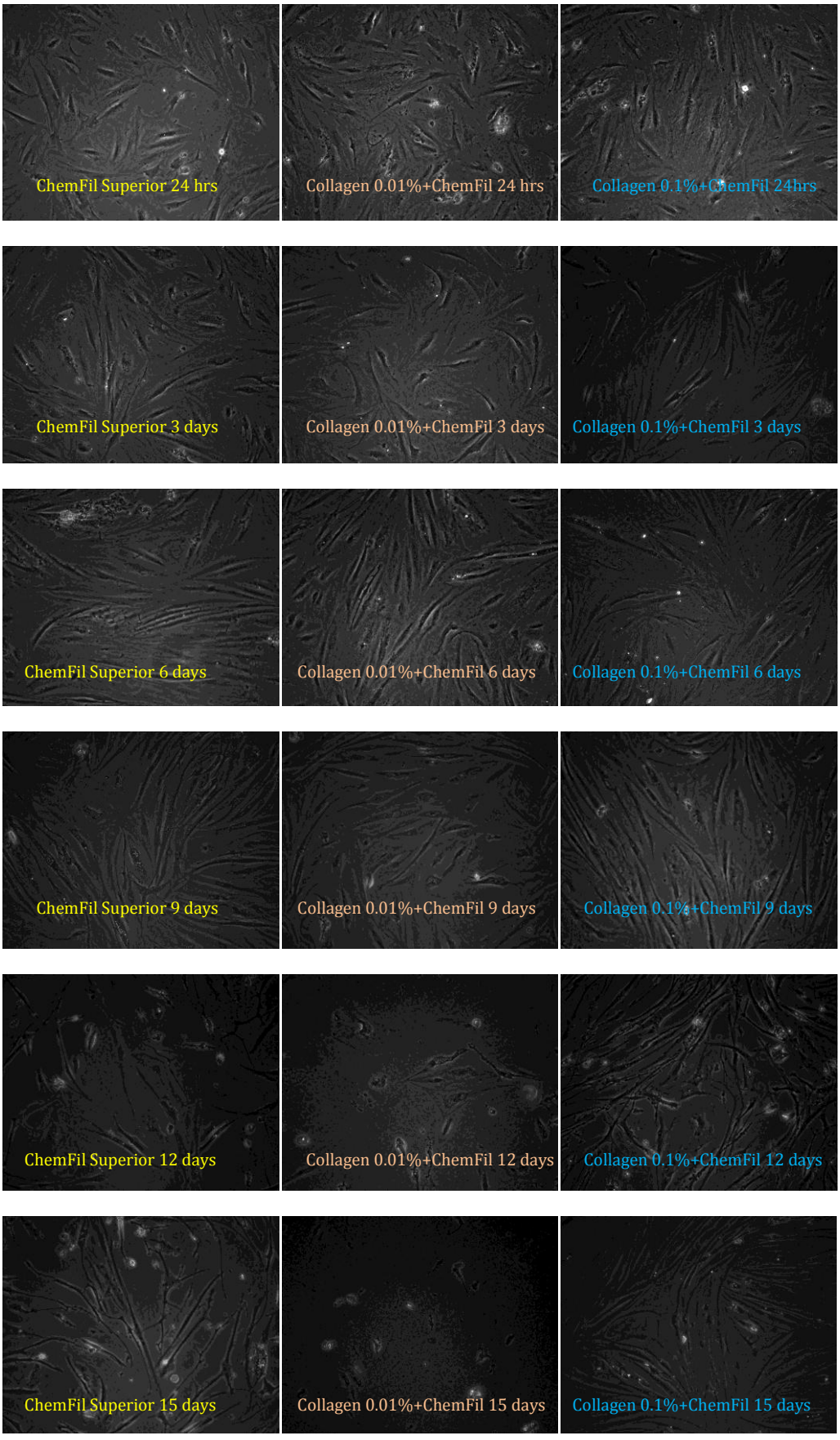


Figure 4-24 The oral mucosa fibroblast cells close to unmodified and bio-modified ChemFil superior with different concentration of Type I collagen for 21 days.


ChemFil Superior 24 hrs
Collagen 0.01%+ChemFil 24 hrs
Collagen 0.1%+ChemFil 24hrs
ChemFil Superior 3 days
Collagen 0.01%+ChemFil 3 days
Collagen 0.1%+ChemFil 3 days
ChemFil Superior 6 days
Collagen 0.01%+ChemFil 6 days
Collagen 0.1%+ChemFil 6 days
ChemFil Superior 9 days
Collagen 0.01%+ChemFil 9 days
Collagen 0.1%+ChemFil 9 days
ChemFil Superior 12 days
Collagen 0.01%+ChemFil 12 days
Collagen 0.1%+ChemFil 12 days
ChemFil Superior 15 days
Collagen 0.01%+ChemFil 15 days
Collagen 0.1%+ChemFil 15 days

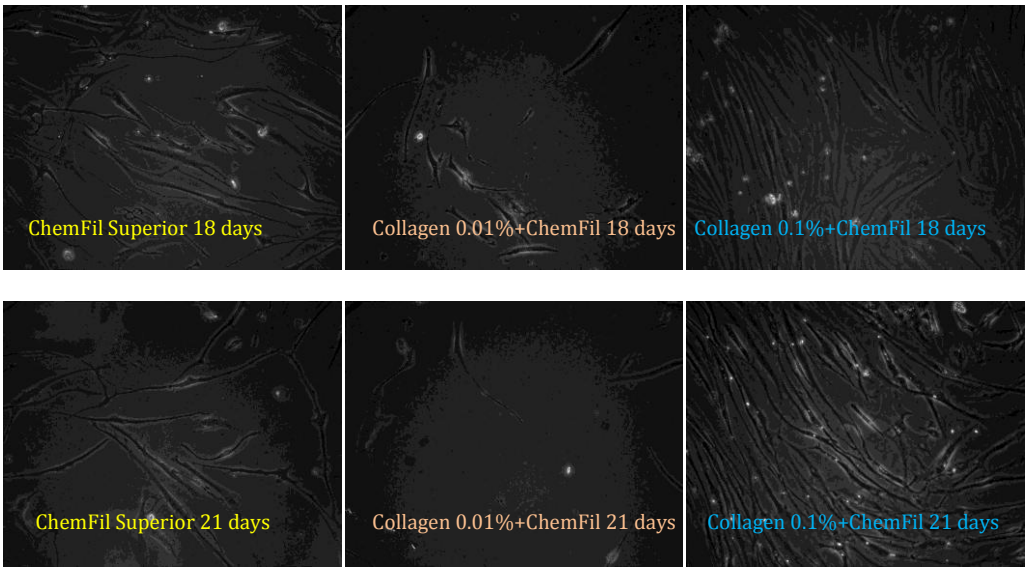


Figure 4-25 The oral mucosa fibroblast cells away from unmodified and bio-modified ChemFil superior with different concentration of Type I collagen for 21 days.

A Kruskal-Wallis test of the pooled rankings of cell viabilities of the cell close and away from ChemFil superior glass ionomer cement modified by the addition of two different concentration of type I collagen (0.01%, 0.1%) and control at all the observed time points demonstrated very highly statistically significant differences (*P < 0.0001*) between the groups. These were localised using a Dunn's Multiple comparison test whose outcome is summarised in tables 4.17-18

Table 4-17 Dunn's Multiple comparison test of rankings of cell viabilities cell close to unmodified and modified ChemFil superior glass ionomer cement by the addition of different concentration of type I collagen (0.01%, 0.1%) .

Versus	0.1% ChemFil Superior + Collagen type I (close)	ChemFil Superior + 0.01% Collagen type I (close)
ChemFil Superior (close)	***	***
ChemFil Superior +0.1% Collagen type I (close)	—	***

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

Table 4-18 Dunn's Multiple comparison test of rankings of cell viabilities cell away from unmodified and modified ChemFil superior glass ionomer cement by the addition of different concentration of type I collagen (0.01%, 0.1%).

Versus	ChemFil Superior +0.1% type I Collagen (Away)	ChemFil Superior + 0.01% type I Collagen (Away)
ChemFil Superior (Away)	NS	*
ChemFil Superior +0.1% type I Collagen (Away)	—	***

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

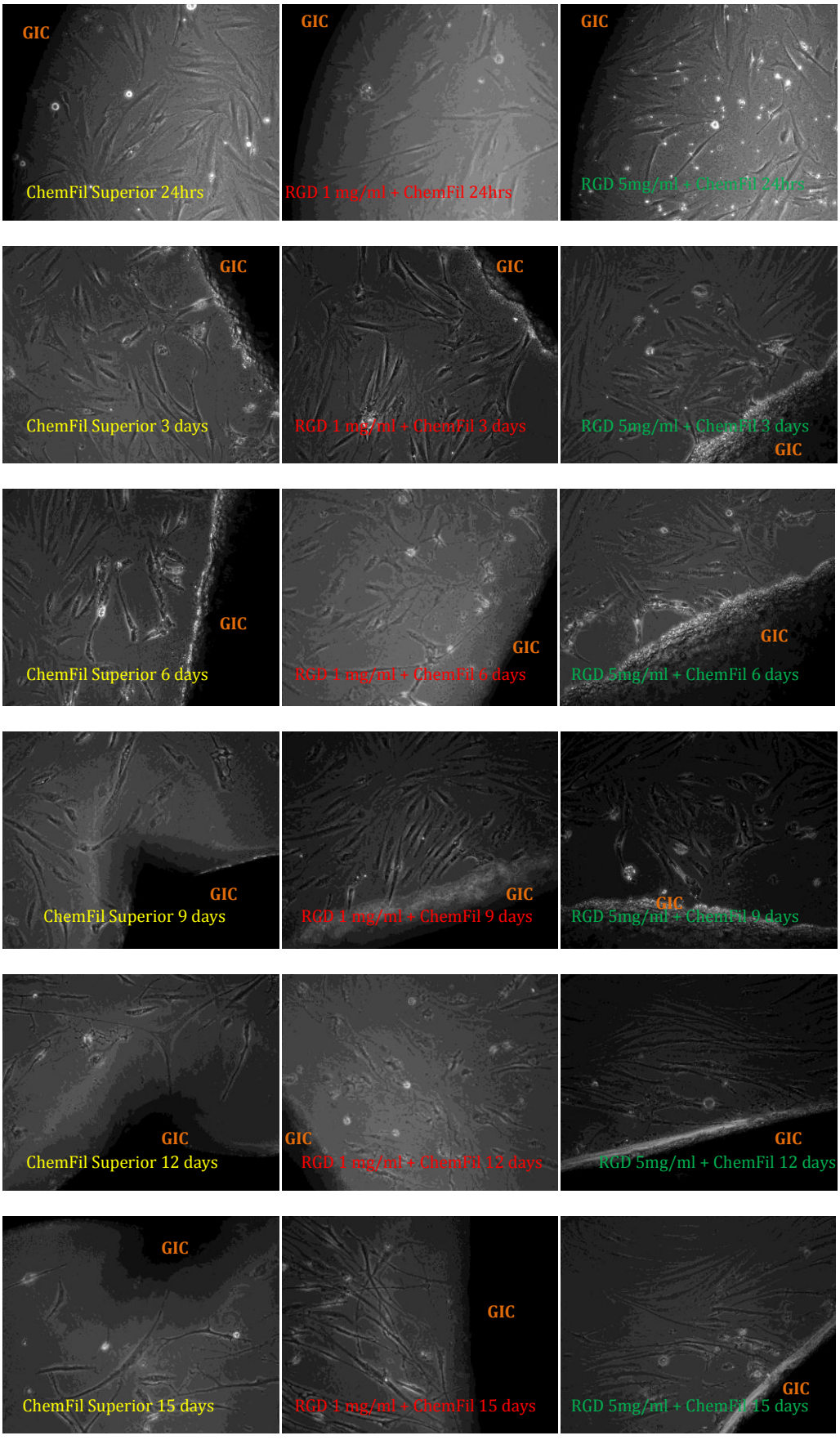
The most common ranking scores for each group were,

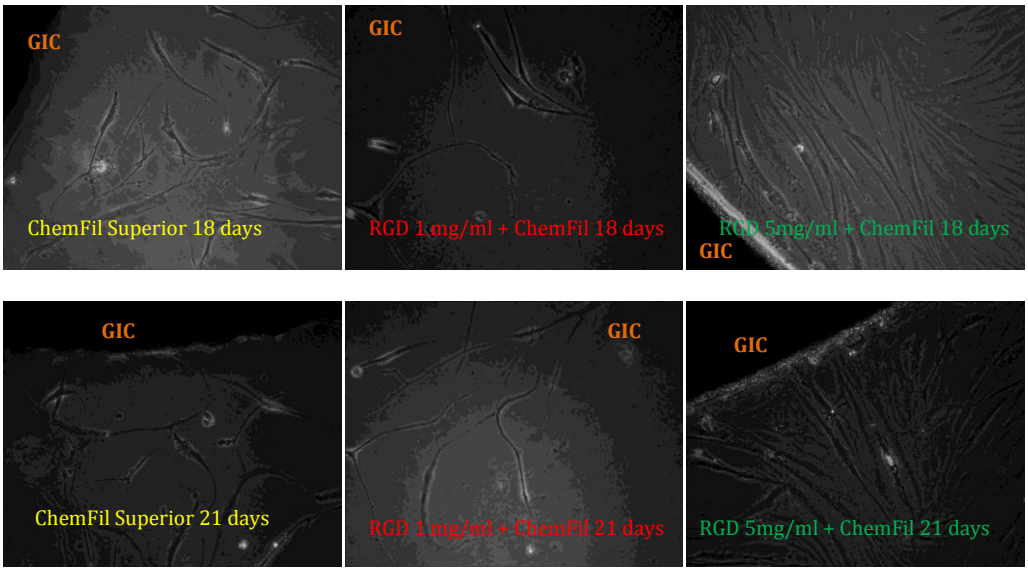
- ChemFil Superior **(close)** = Rank 3 (Range = 3, Min Rank = 1, Max Rank = 4).
- ChemFil Superior +0.1% Type I Collagen **(Close)** = Rank 2 (Range = 2, Min Rank = 1, Max Rank = 3).
- ChemFil Superior +0.01% Type I Collagen **(Close)** = Rank 3 (Range = 3, Min Rank =1, Max Rank = 4).
- ChemFil Superior **(Away)** = Rank 3 (Range = 3, Min Rank = 1, Max Rank = 4).
- ChemFil Superior +0.1% Type I Collagen **(Away)** = Rank 2 (Range = 2, Min Rank = 1, Max Rank = 3)
- ChemFil Superior +0.01% Type I Collagen **(Away)** = Rank 4 (Range = 3, Min Rank =1, Max Rank = 4)

It is thus evident that the highest cell viability was associated with ChemFil Superior modified with 0.1% Type I Collagen.

- **RGD OPTIMAL CONCENTRATION**

The oral mucosa fibroblast cells cultured around unmodified and bio-modified ChemFil superior with different concentration of RGD for 21 days. The pictures to the cells were taken every 3 days close and away to the specimens Figure 4-26 and 4-27.


GIC
ChemFil Superior 24hrs
GIC
RGD 1 mg/ml + ChemFil 24hrs
GIC
RGD 5mg/ml + ChemFil 24hrs
GIC
ChemFil Superior 3 days
GIC
RGD 1 mg/ml + ChemFil 3 days
RGD 5mg/ml + ChemFil 3 days
GIC
GIC
ChemFil Superior 6 days
GIC
RGD 1 mg/ml + ChemFil 6 days
GIC
RGD 5mg/ml + ChemFil 6 days
GIC
ChemFil Superior 9 days
GIC
RGD 1 mg/ml + ChemFil 9 days
GIC
RGD 5mg/ml + ChemFil 9 days
GIC
ChemFil Superior 12 days
GIC
RGD 1 mg/ml + ChemFil 12 days
GIC
RGD 5mg/ml + ChemFil 12 days
GIC
ChemFil Superior 15 days
GIC
RGD 1 mg/ml + ChemFil 15 days
GIC
RGD 5mg/ml + ChemFil 15 days



4-26 The oral mucosa fibroblast cells close to unmodified and bio-modified ChemFil superior with different concentration of RGD for 21 days.

A Kruskal-Wallis test of the pooled rankings of cell viabilities of oral mucosal cells, close to unmodified ChemFil superior glass ionomer cement and modified ChemFil superior glass ionomer cement, by the addition of two different concentration of RGD (1mg/ml, 5mg/ml) seen at all observed time points demonstrated very highly statistically significant differences (*P < 0.0001*) between the groups. These were localised using a Dunn's Multiple comparison test whose outcome is summarised in table 4-19

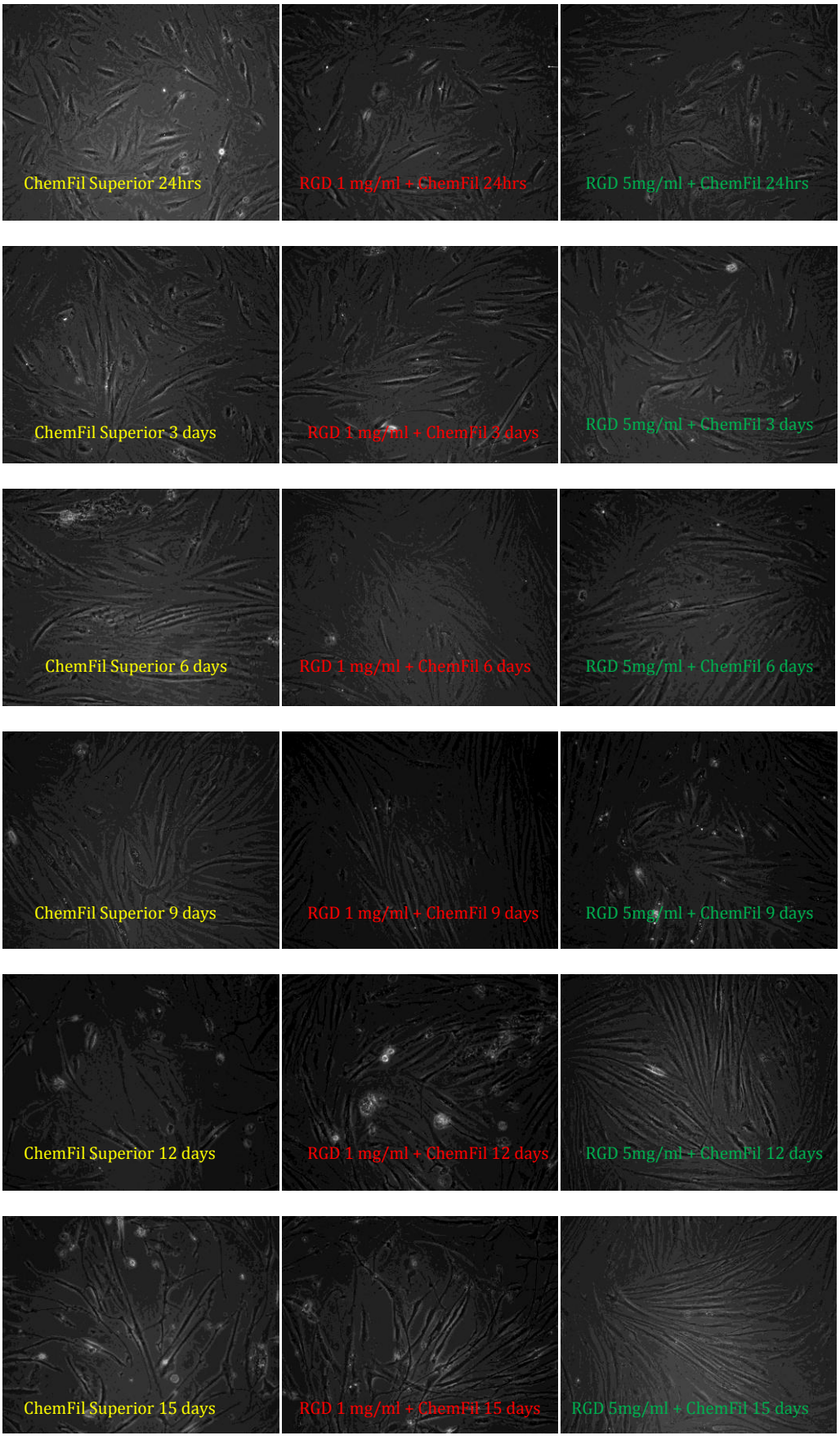
Table 4-19 Dunn's Multiple comparison test of rankings of cell viabilities cell close to unmodified and modified ChemFil superior glass ionomer cement by the addition of two different concentration of RGD (1mg/ml, 5mg/ml).

Versus	ChemFil Superior + RGD 1mg/ml (close)	ChemFil Superior + RGD 5ml/ml (close)
ChemFil Superior (close)	NS	***
ChemFil Superior +RGD 5mg/ml (close)	***	—

Key; NS = not significant, * = p < 0.05, **= p < 0.001, *** = p < 0.0001

The most common ranking scores for each group were,

- ChemFil Superior **(close)** = Rank 3 (Range = 3, Min Rank = 1, Max Rank = 4).
- ChemFil Superior + RGD 1mg/ml **(Close)** = Rank 3 (Range = 3, Min Rank = 1, Max Rank = 3).
- ChemFil Superior +RGD 5mg/ml **(Close)** = Rank 2 (Range = 2, Min Rank =1, Max Rank = 3).


ChemFil Superior 24hrs
RGD 1 mg/ml + ChemFil 24hrs
RGD 5mg/ml + ChemFil 24hrs
ChemFil Superior 3 days
RGD 1 mg/ml + ChemFil 3 days
RGD 5mg/ml + ChemFil 3 days
ChemFil Superior 6 days
RGD 1 mg/ml + ChemFil 6 days
RGD 5mg/ml + ChemFil 6 days
ChemFil Superior 9 days
RGD 1 mg/ml + ChemFil 9 days
RGD 5mg/ml + ChemFil 9 days
ChemFil Superior 12 days
RGD 1 mg/ml + ChemFil 12 days
RGD 5mg/ml + ChemFil 12 days
ChemFil Superior 15 days
RGD 1 mg/ml + ChemFil 15 days
RGD 5mg/ml + ChemFil 15 days

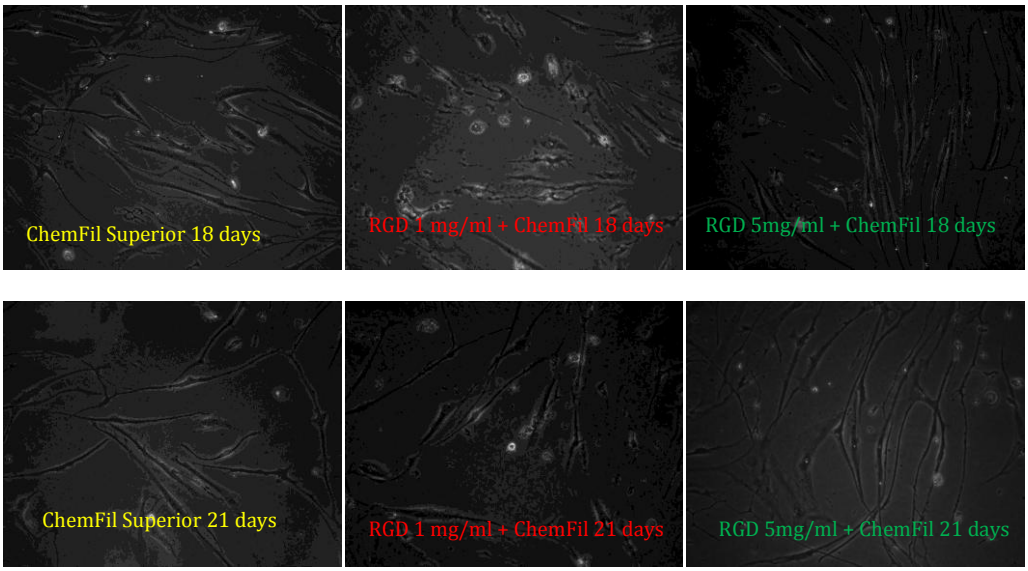


Figure 4-27 The oral mucosa fibroblast cells away from unmodified and bio-modified ChemFil superior with different concentration of RGD for 21 days.

A Kruskal-Wallis test of the pooled rankings of cell viabilities of oral mucosal cells, away from unmodified ChemFil superior glass ionomer cement and modified ChemFil superior glass ionomer cement, by the addition of two different concentration of RGD (1mg/ml, 5mg/ml) seen at all observed time points demonstrated highly statistically significant differences ($P < 0.001$) between the groups. These were localised using a Dunn's Multiple comparison test whose outcome is summarised in table 4-20

Table 4-20 Dunn's Multiple comparison test of rankings of cell viabilities cell away from unmodified and modified ChemFil superior glass ionomer cement by the addition of two different concentration of RGD (1mg/ml, 5mg/ml).

Versus	ChemFil Superior + RGD 1mg/ml (Away)	ChemFil Superior + RGD 5ml/ml (Away)
ChemFil Superior (Away)	NS	**
ChemFil Superior +RGD 5mg/ml (Away **)**	*	—

Key; NS = not significant, * = p < 0.05, **= p < 0.001, *** = p < 0.0001

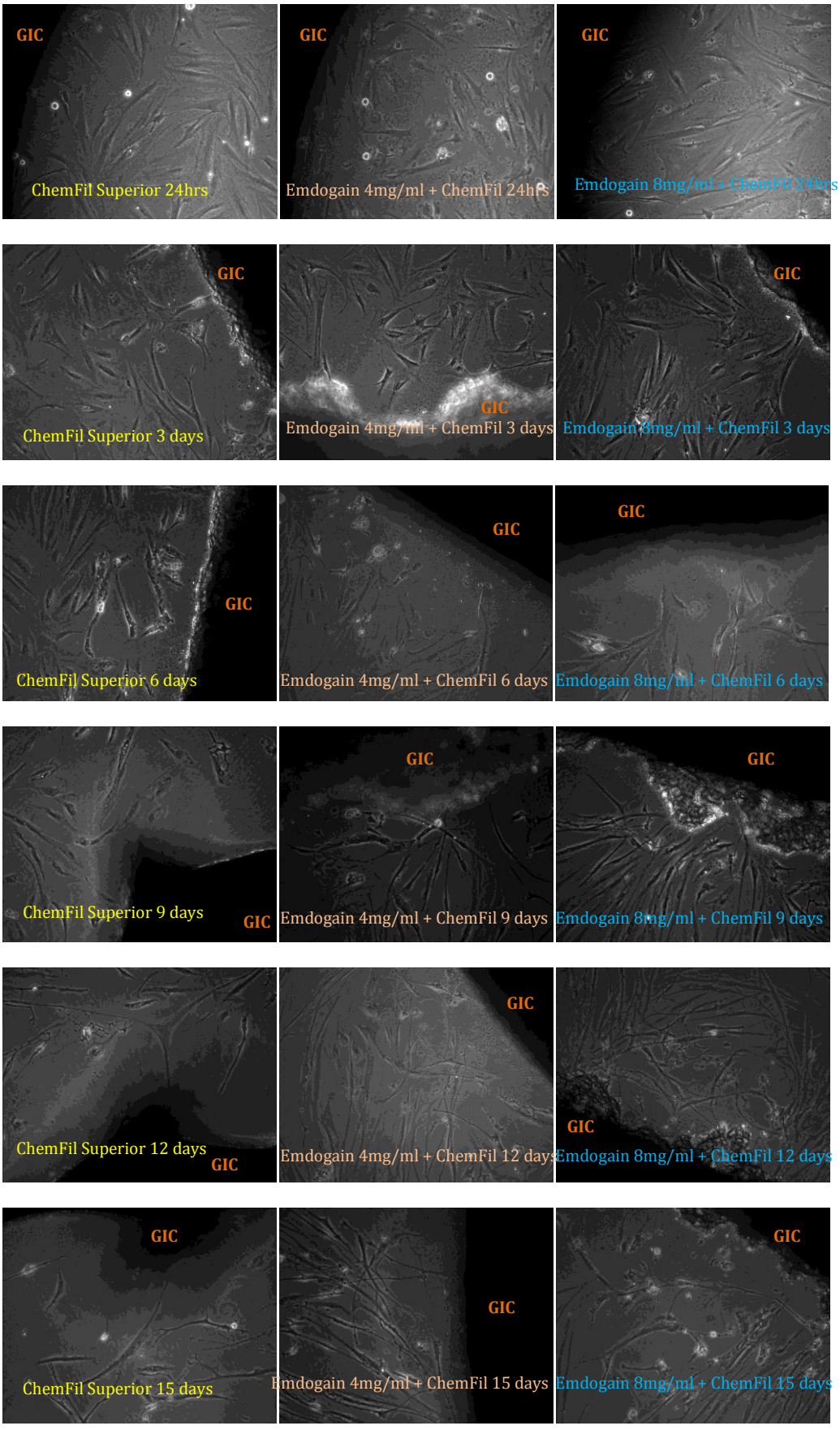
The most common ranking scores for each group were,

- ChemFil Superior **(Away)** = Rank 2(Range = 3, Min Rank = 1, Max Rank = 4).
- ChemFil Superior + RGD 1mg/ml **(Away)** = Rank 2 (Range = 3, Min Rank = 1, Max Rank = 4).
- ChemFil Superior +RGD 5mg/ml **(Away)** = Rank 2 (Range =3, Min Rank =1, Max Rank = 4).

It is thus evident that the highest cell viability was associated with ChemFil Superior modified with 5mg/ml RGD.

- **EMDOGAIN OPTIMAL CONCENTRATION**

The oral mucosa fibroblast cells cultured around unmodified and bio-modified ChemFil superior with different concentration of RGD for 21 days. The pictures to the cells were taken every 3 days close and away to the specimens Figure 4-28 and 4-29.


GIC
ChemFil Superior 24hrs
GIC
Emdogain 4mg/ml + ChemFil 24hrs
GIC
Emdogain 8mg/ml + ChemFil 24hrs
GIC
ChemFil Superior 3 days
GIC
Emdogain 4mg/ml + ChemFil 3 days
GIC
Emdogain 8mg/ml + ChemFil 3 days
GIC
ChemFil Superior 6 days
GIC
Emdogain 4mg/ml + ChemFil 6 days
GIC
Emdogain 8mg/ml + ChemFil 6 days
ChemFil Superior 9 days
GIC
GIC
Emdogain 4mg/ml + ChemFil 9 days
GIC
Emdogain 8mg/ml + ChemFil 9 days
ChemFil Superior 12 days
GIC
GIC
Emdogain 4mg/ml + ChemFil 12 days
GIC
Emdogain 8mg/ml + ChemFil 12 days
GIC
ChemFil Superior 15 days
GIC
Emdogain 4mg/ml + ChemFil 15 days
GIC
Emdogain 8mg/ml + ChemFil 15 days

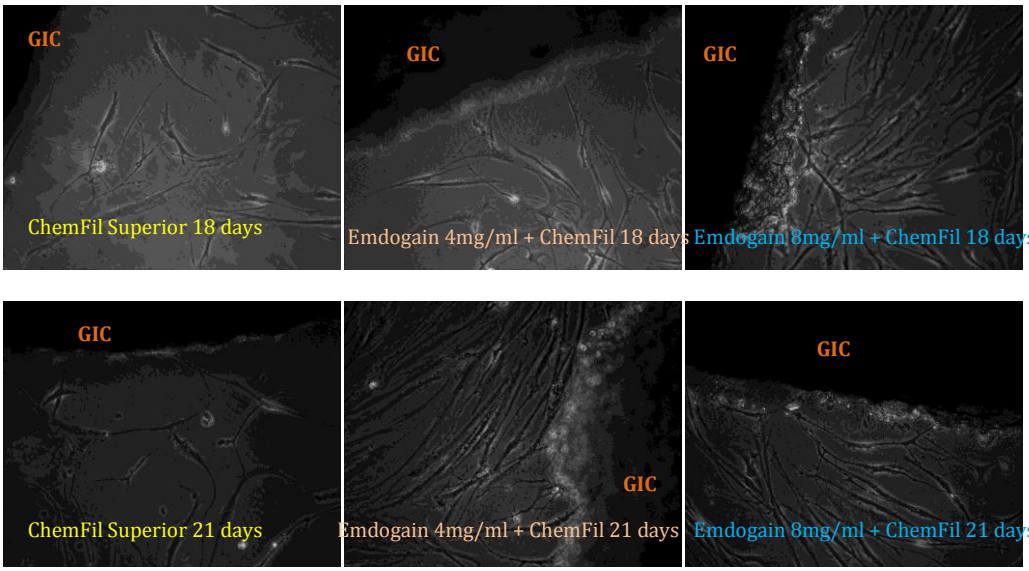


Figure 4-28 The oral mucosa fibroblast cells close to bio-modified ChemFil superior with different concentration of Emdogain for 21 days.

A Kruskal-Wallis test of the pooled rankings of cell viabilities of oral mucosal cells, close to unmodified ChemFil superior glass ionomer cement and modified ChemFil superior glass ionomer cement, by the addition of two different concentration of Emdogain (4mg/ml, 8mg/ml) seen over all observation times demonstrated very statistically significant differences ($P < 0.05$) between the groups. These were localised using a Dunn's Multiple comparison test whose outcome is summarised in table 4-21

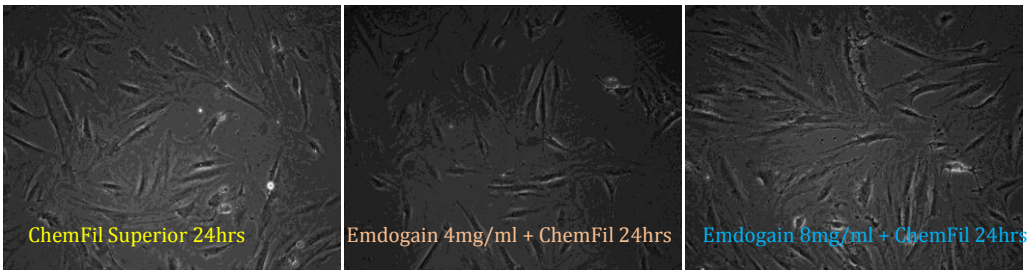
Table 4-21 Dunn's Multiple comparison test of rankings of cell viabilities cell close to unmodified and modified ChemFil superior glass ionomer cement by the addition of two different concentration of Emdogain (4mg/ml, 8mg/ml).

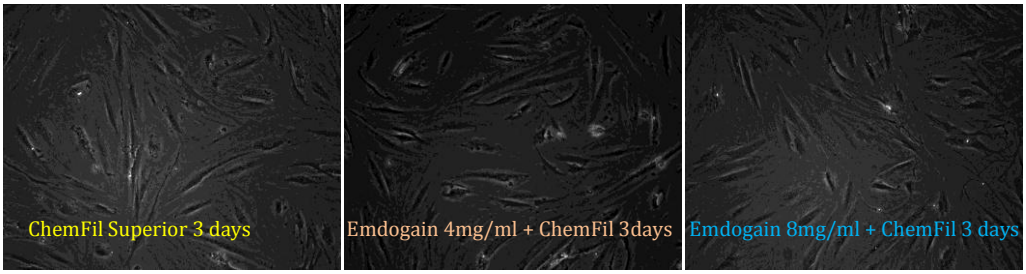
Versus	ChemFil Superior + Emdogain 4mg/ml (close)	ChemFil Superior + Emdogain 8ml/ml (close)
ChemFil Superior (close)	NS	**
ChemFil Superior +Emdogain 8mg/ml (close)	NS	—

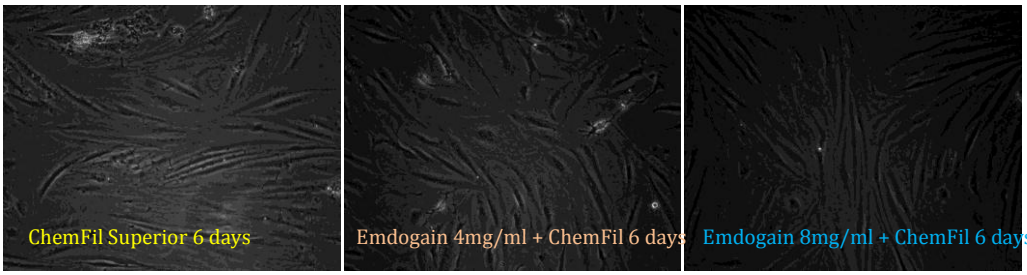
Key; NS = not significant, * = p < 0.05, **= p < 0.001, *** = p < 0.0001

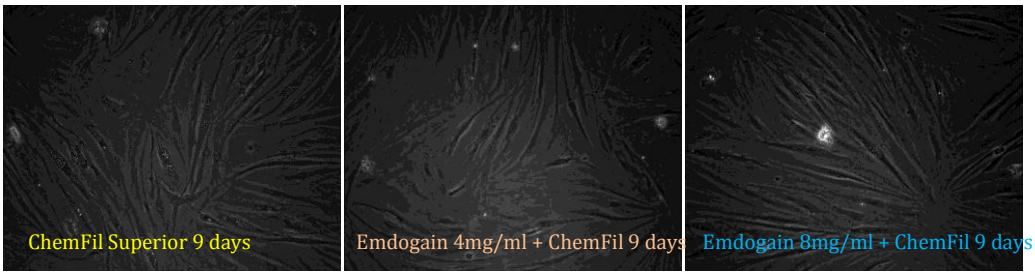
The most common ranking scores for each group were,

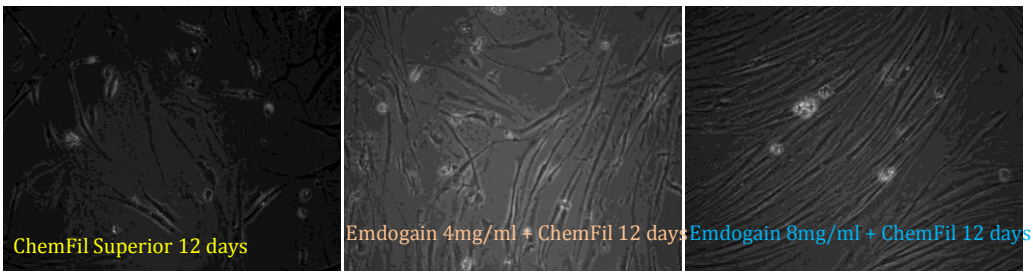
- ChemFil Superior **(close)** = Rank 3 (Range = 3, Min Rank = 1, Max Rank = 4).
- ChemFil Superior + Emdogain 4mg/ml **(Close)** = Rank 3 (Range = 3, Min Rank = 1, Max Rank = 4).
- ChemFil Superior +Emdogain 8mg/ml **(Close)** = Rank 3 (Range = 3, Min Rank =1, Max Rank = 4).

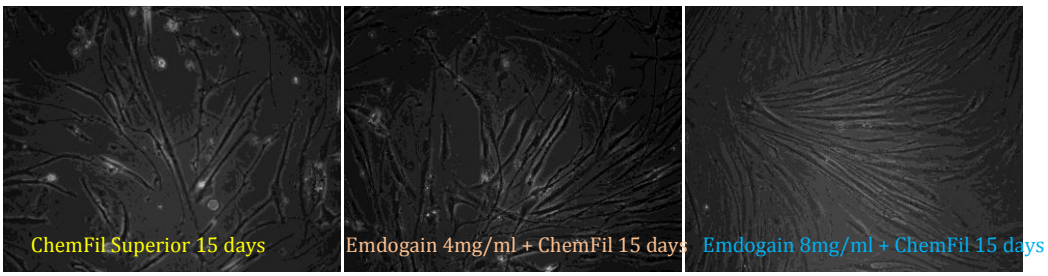

ChemFil Superior 24hrs
Emdogain 4mg/ml + ChemFil 24hrs
Emdogain 8mg/ml + ChemFil 24hrs


ChemFil Superior 3 days
Emdogain 4mg/ml + ChemFil 3days
Emdogain 8mg/ml + ChemFil 3 days


ChemFil Superior 6 days
Emdogain 4mg/ml + ChemFil 6 days
Emdogain 8mg/ml + ChemFil 6 days


ChemFil Superior 9 days
Emdogain 4mg/ml + ChemFil 9 days
Emdogain 8mg/ml + ChemFil 9 days


ChemFil Superior 12 days
Emdogain 4mg/ml + ChemFil 12 days
Emdogain 8mg/ml + ChemFil 12 days


ChemFil Superior 15 days
Emdogain 4mg/ml + ChemFil 15 days
Emdogain 8mg/ml + ChemFil 15 days

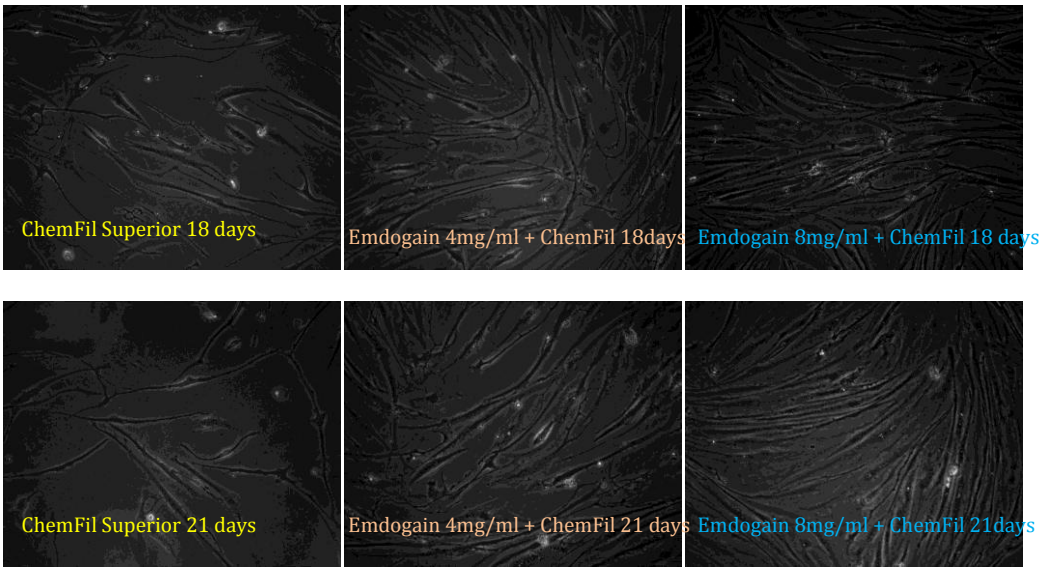


Figure 4-29 The oral mucosa fibroblast cells away from unmodified and bio-modified ChemFil superior with different concentration of Emdogain for 21.

A Kruskal-Wallis test of the pooled rankings of cell viabilities of oral mucosal cells, located away from unmodified ChemFil superior glass ionomer cement and modified ChemFil superior glass ionomer cement, by the addition of two different concentration of Emdogain (4mg/ml, 8mg/ml) seen at all observed time points demonstrated very highly statistically significant differences (*P < 0.001*) between the groups. These were localised using a Dunn's Multiple comparison test whose outcome is summarised in table 4-22

Table 4-22 Dunn's Multiple comparison test of rankings of cell viabilities cell located away from unmodified and modified ChemFil superior glass ionomer cement by the addition of two different concentration of Emdogain (4mg/ml, 8mg/ml).

Versus	ChemFil Superior + Emdogain 4mg/ml (Away)	ChemFil Superior + Emdogain 8ml/ml (Away)
ChemFil Superior (Away)	NS	***
ChemFil Superior +Emdogain 8mg/ml (Away)	***	—

Key; NS = not significant, * = p < 0.05, **= p < 0.001, *** = p < 0.0001

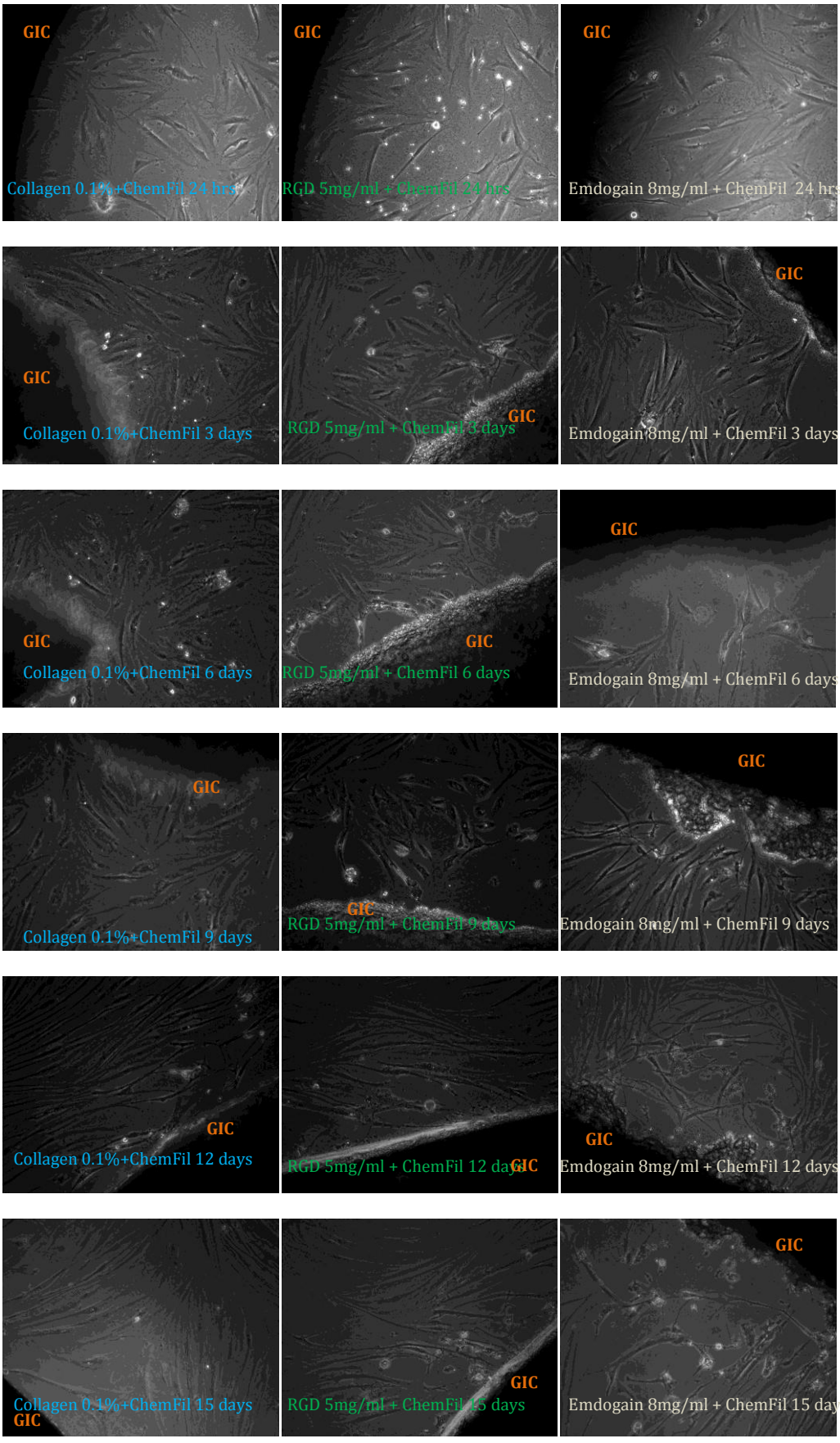
The most common ranking scores for each group were,

- ChemFil Superior **(Away)** = Rank 2 (Range = 3, Min Rank = 1, Max Rank = 4).
- ChemFil Superior + Emdogain 4mg/ml **(Away)** = Rank 2 (Range = 3, Min Rank = 1, Max Rank = 4).
- ChemFil Superior +Emdogain 8mg/ml **(Away)** = Rank 2 (Range = 2, Min Rank =1, Max Rank = 3).

It is thus evident that better cell viability was associated with ChemFil Superior modified with 8mg/ml Emdogain.

4.2.1.1.3 DETERMINATION OF OPTIMAL BIOACTIVE ADDITIVES

The oral mucosa fibroblast cells cultured around bio-modified ChemFil superior with three different Bio-additives for 21 days. The pictures to the cells were taken every 3 days close and away to the specimens Figure 4-30 and 4-31.


GIC
Collagen 0.1%+ChemFil 24 hrs
GIC
RGD 5mg/ml + ChemFil 24 hrs
GIC
Emdogain 8mg/ml + ChemFil 24 hrs
GIC
Collagen 0.1%+ChemFil 3 days
GIC
RGD 5mg/ml + ChemFil 3 days
GIC
Emdogain 8mg/ml + ChemFil 3 days
GIC
Collagen 0.1%+ChemFil 6 days
GIC
RGD 5mg/ml + ChemFil 6 days
GIC
Emdogain 8mg/ml + ChemFil 6 days
GIC
Collagen 0.1%+ChemFil 9 days
GIC
RGD 5mg/ml + ChemFil 9 days
GIC
Emdogain 8mg/ml + ChemFil 9 days
GIC
Collagen 0.1%+ChemFil 12 days
GIC
RGD 5mg/ml + ChemFil 12 days
GIC
Emdogain 8mg/ml + ChemFil 12 days
Collagen 0.1%+ChemFil 15 days
GIC
GIC
RGD 5mg/ml + ChemFil 15 days
GIC
Emdogain 8mg/ml + ChemFil 15 days

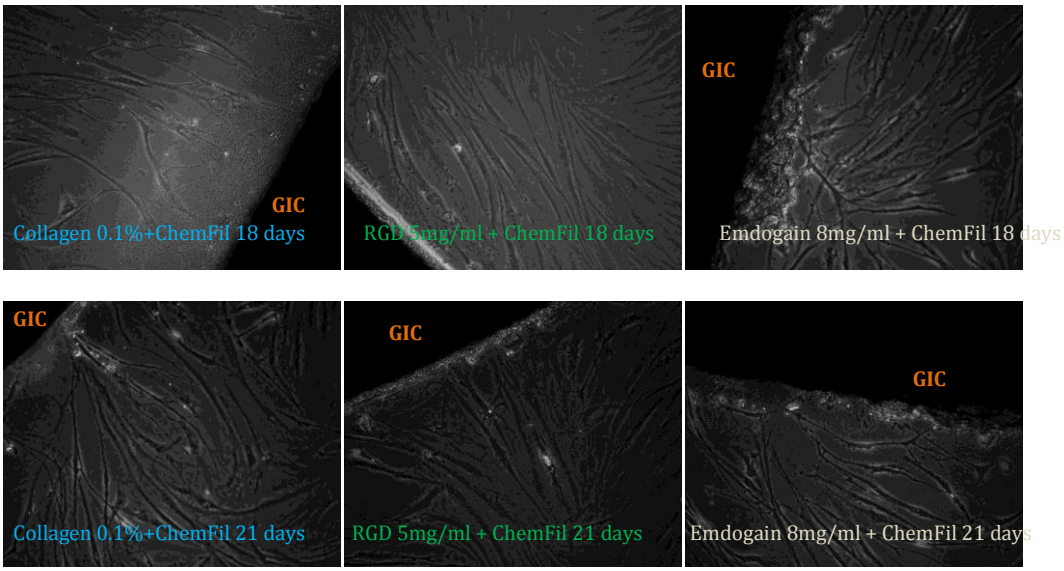


Figure 4-30 The oral mucosa fibroblast cells close to bio-modified ChemFil superior with 3 different bio-additive for 21 days.

A Kruskal-Wallis test of the pooled rankings of cell viabilities of oral mucosal cells, close to modified ChemFil superior glass ionomer cement (0.1% Collagen type I, RGD 5mg/ml, Emdogain 8mg/ml) seen at all observed time points demonstrated very highly statistically significant differences (*P < 0.0001*) between the groups. These were localised using a Dunn's Multiple comparison test whose outcome is summarised in table 4-23.

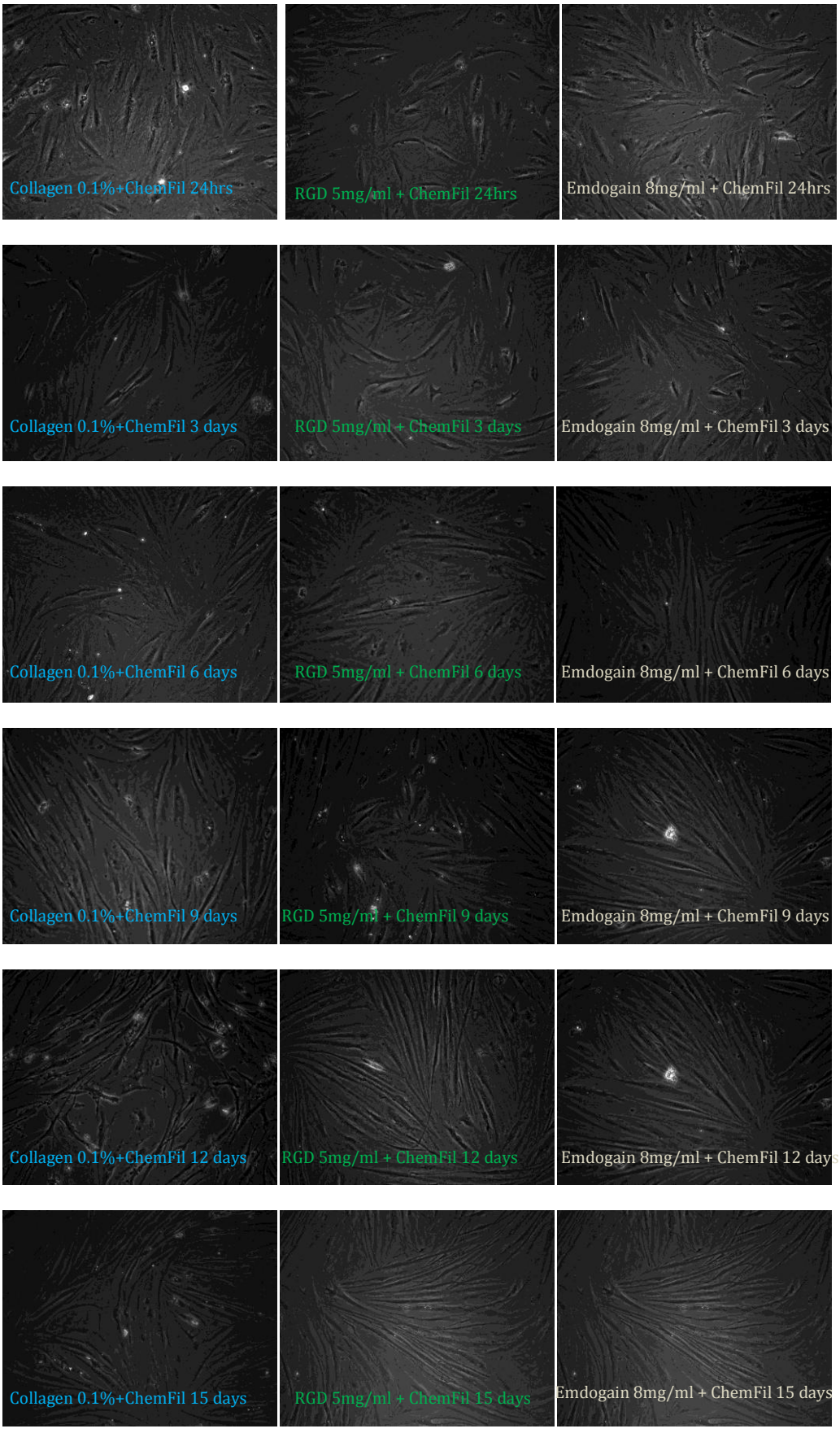
Table 4-23 Dunn's Multiple comparison test of rankings of cell viabilities cell close to unmodified and modified ChemFil superior glass ionomer cement by the addition Type I collagen, RGD and, Emdogain.

Versus	ChemFil Superior +0.1% Collagen type I (close)	ChemFil Superior + Emdogain 8ml/ml (close)
ChemFil Superior +RGD 5mg/ml (close)	NS	***
ChemFil Superior +Emdogain 8mg/ml (close)	***	—

Key; NS = not significant, * = p < 0.05, **= p < 0.001, *** = p < 0.0001

The most common ranking scores for each group were,

- ChemFil Superior+ 0.1% Collagen type I **(close)** = Rank 2 (Range = 3, Min Rank = 1, Max Rank = 4).
- ChemFil Superior + RGD 5mg/ml **(Close)** = Rank 2 (Range = 1, Min Rank = 2, Max Rank = 3).
- ChemFil Superior +Emdogain 8mg/ml **(Close)** = Rank 3 (Range = 3, Min Rank =1, Max Rank = 4).


Collagen 0.1%+ChemFil 24hrs
RGD 5mg/ml + ChemFil 24hrs
Emdogain 8mg/ml + ChemFil 24hrs
Collagen 0.1%+ChemFil 3 days
RGD 5mg/ml + ChemFil 3 days
Emdogain 8mg/ml + ChemFil 3 days
Collagen 0.1%+ChemFil 6 days
RGD 5mg/ml + ChemFil 6 days
Emdogain 8mg/ml + ChemFil 6 days
Collagen 0.1%+ChemFil 9 days
RGD 5mg/ml + ChemFil 9 days
Emdogain 8mg/ml + ChemFil 9 days
Collagen 0.1%+ChemFil 12 days
RGD 5mg/ml + ChemFil 12 days
Emdogain 8mg/ml + ChemFil 12 days
Collagen 0.1%+ChemFil 15 days
RGD 5mg/ml + ChemFil 15 days
Emdogain 8mg/ml + ChemFil 15 days

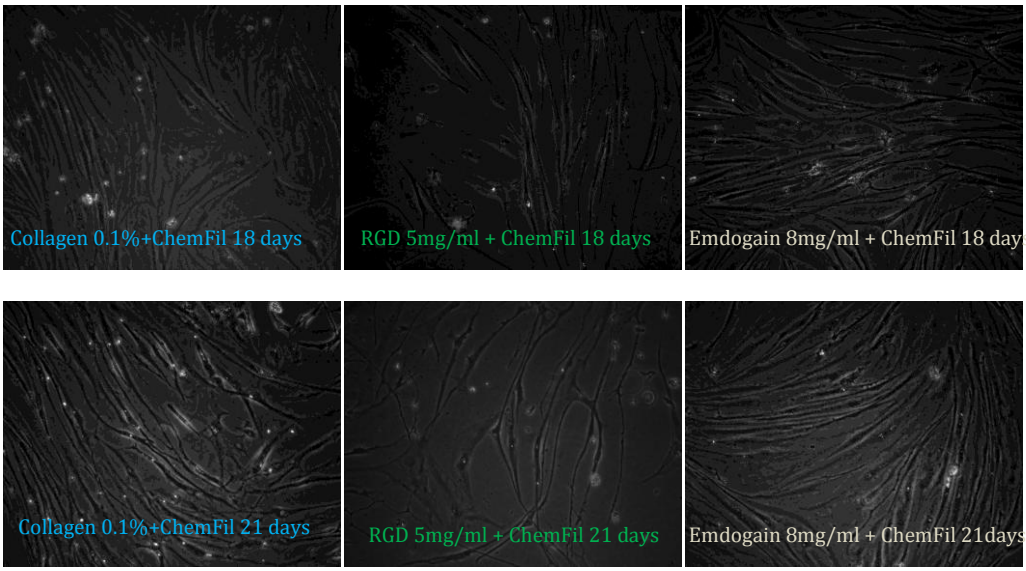


Figure 4-31 The oral mucosa fibroblast cells away from unmodified and bio-modified ChemFil superior with different bio-additives for 21 days specimens for 21 days.

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A Kruskal-Wallis test of the pooled rankings of cell viabilities of oral mucosal cells, located away from modified ChemFil superior glass ionomer cement (0.1% Collagen type I, RGD 5mg/ml, Emdogain 8mg/ml) seen at all observed time points demonstrated statistically significant differences ($P < 0.05$) between the groups. These were localised using a Dunn's Multiple comparison test whose outcome is summarised in table 4.24

Table 4-24 Dunn's Multiple comparison test of rankings of cell viabilities cell close to unmodified and modified ChemFil superior glass ionomer cement by the addition of Type I collagen, RGD and, Emdogain.

Versus	ChemFil Superior +0.1% Collagen type I (Away)	ChemFil Superior + Emdogain 8ml/ml (Away)
ChemFil Superior +RGD 5mg/ml (Away)	NS	**
ChemFil Superior +Emdogain 8mg/ml (Away)	NS	—

Key; NS = not significant, * = p < 0.05, **= p < 0.001, *** = p < 0.0001

The most common ranking scores for each group were,

- ChemFil Superior+ 0.1% Collagen type I **(Away)** = Rank 2 (Range = 2, Min Rank = 1, Max Rank = 3).
- ChemFil Superior + RGD 5mg/ml **(Away)** = Rank 2 (Range =3, Min Rank =1, Max Rank = 4).
- ChemFil Superior +Emdogain 8mg/ml **(Away)** = Rank 1 (Range = 3, Min Rank =1, Max Rank = 4).

4.2.1.2 *CELL VIABILITY TESTING (MTT ASSAY)*

4.2.1.2.1 DETERMINATION OF OPTIMAL MATERIAL FOR ATTACHMENT OF CELLS

To determine the effect of GC Fuji VIII and ChemFil superior glass ionomer cements on oral mucosa fibroblast viability MTT Assays were carried out. The viability of the oral mucosa was measured after 24 hour and 72 hours from culturing oral mucosa fibroblast cells with those materials.

The absorbance values obtained for each well represent the amount of MTT reduction, which is proportional to the number of viable cells. In order to assess the percentage of viable cells present in each well, the absorbance values were related to those of the control. This was achieved by setting the mean absorbance of the control at 100%.

The percentages of the effect of ChemFil Superior and GC Fuji VIII on cell viability after 24 and 72 hours are presented in tables 4-25 and 4-26 respectively.

Table 4-25 Percentages of cell viability relative to the control (100% viability) after direct contact with the materials after 24 hours.

Sample	Fibroblast Cells	ChemFil Superior	GC Fuji VIII
1	105.54	33.95	22.80
2	90.04	12.92	36.26
3	104.43	46.13	38.74
4	95.33	40.59	31.32
5	99.73	24.72	35.71
6	104.67	54.24	30.22
7	60.07	39.85	22.80
8	112.15	33.58	34.07
9	127.43	38.01	54.40
Mean	99.93	**36.00**	**34.04**
SD	18.33	11.96	9.48

Table 4-26 Percentages of cell viability relative to the control (100% viability) after direct contact with the materials after 72 hours.

Sample	Fibroblast Cells	ChemFil Superior	GC Fuji VIII
1	103.92	9.70	0.38
2	91.91	17.06	3.01
3	104.44	9.70	1.50
4	51.13	10.37	4.51
5	122.18	10.70	2.63
6	126.69	5.69	3.38
7	75.25	14.72	1.13
8	129.43	8.36	2.26
9	95.32	8.36	0.38
Mean	**100.00**	**2.13**	**10.52**
SD	25.48	1.05	3.45

On the first 24 hours, cells exposed to both ChemFil Superior and GC Fuji VIII showed similar viability around 35 %. After 72 hours the average percentages of the viability of the cells has been markedly reduced for both materials i.e. (ChemFil superior 10.52% and GC Fuji VIII 2.13%) (Figure 4.32).

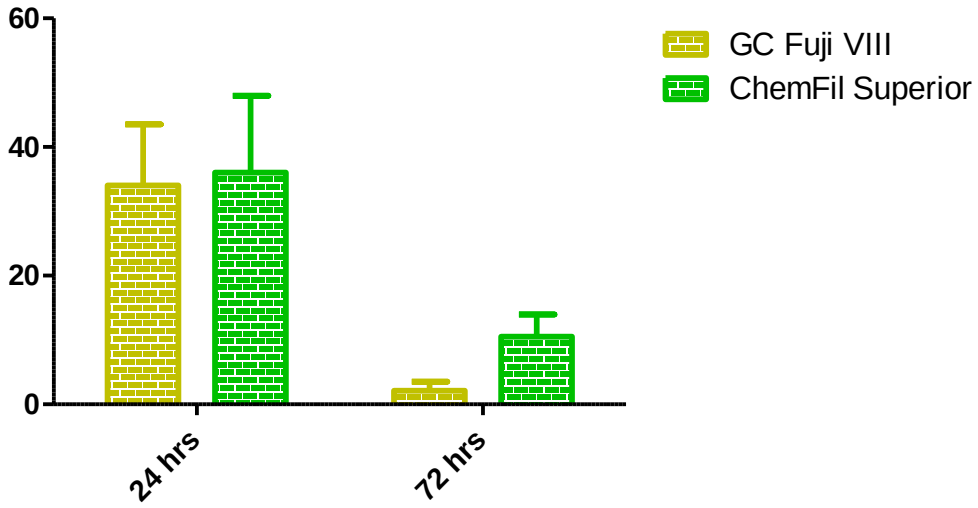

Figure 4-32 Percentage of cell viability (Mean+ SD) relative to the control (100% viability) for materials after 24 and 72 hours.

Statistical analysis (Unpaired t test) showed that no significant difference between cells viabilities for ChemFil superior and GC Fuji after 24 hours. However, there was very highly significant deference between both materials after 72 hours ($P < 0.0001$).

It is thus evident that the lowest cell viability was associated with GC Fuji VIII

4.2.1.2.2 DETERMINATION THE OPTIMAL CONCENTRATION OF BIOACTIVE ADDITIVES

To determine the optimal concentration of the bio-additives to ChemFil superior glass ionomer and their effect on cell viability, MTT Assays were carried out after 24 hour and 72 hours. ChemFil Superior glass ionomer cement was modified by three different bio- additives (type I Collagen, RGD, Emdogain) at two different concentrations each. The Absorbance values obtained for each well represent the amount of MTT reduction, which is proportional to the number of viable cells. In order to assess the percentage of viable cells present in each well, the absorbance values were related to those of the control (unmodified ChemFil Superior). This was achieved by setting the mean absorbance of the control at 100%.

The effects of the addition of two different concentrations of type I Collagen (10µg/ml, 100µg/ml) to ChemFil Superior on cell viability after 24 hours and 72 hours are presented in tables 4-27-4-28.

Table 4-27 Percentages of cell viability relative to the unmodified ChemFil Superior (100% viability) after direct contact with the modified ChemFil superior after 24 hours.

Sample	ChemFil Superior 24hrs	Type I Collagen 0.01% 24hrs	Type I Collagen 0.1% 24 hrs
1	95.77	87.24	80.52
2	140.03	97.11	88.31
3	64.14	95.37	57.15
4	98.36	117.80	49.70
5	80.33	92.34	105.51
6	120.49	104.30	50.10
7	69.29	60.66	60.66
8	113.28	85.25	85.25
9	117.84	63.93	63.93
Mean	**99.95**	**89.33**	**71.24**
SD	47.2	18.13	19.44

Table 4-28 Percentages of cell viability relative to the control (100% viability) after direct contact with modified ChemFil superior after 72 hours.

Sample	ChemFil Superior	Type I Collagen 0.01%	Type I Collagen 0.1%
1	137.76	200.70	117.48
2	111.19	104.55	361.89
3	50.70	150.00	218.18
4	97.56	176.22	167.83
5	70.73	337.41	65.03
6	131.71	225.87	144.76
7	40.00	78.05	151.22
8	186.67	37.80	60.98
9	66.67	9.76	118.29
Mean	**99.22**	**146.70**	**156.20**
SD	47.52	102.2	91.35

After 24 hours, cells exposed to modified ChemFil Superior with 0.1 % Type I collagen and 0.01% Type I collagen showed less viability than the control around 89.9% and 71.24% respectively. After 72 hours, the average percentages of the viability of the cells has been markedly increased for both materials i.e. (0.01% Type I collagen +ChemFil superior 146.7% and 0.1 %Type I collagen +ChemFil superior 156.6.2%) (Figure 4-33).

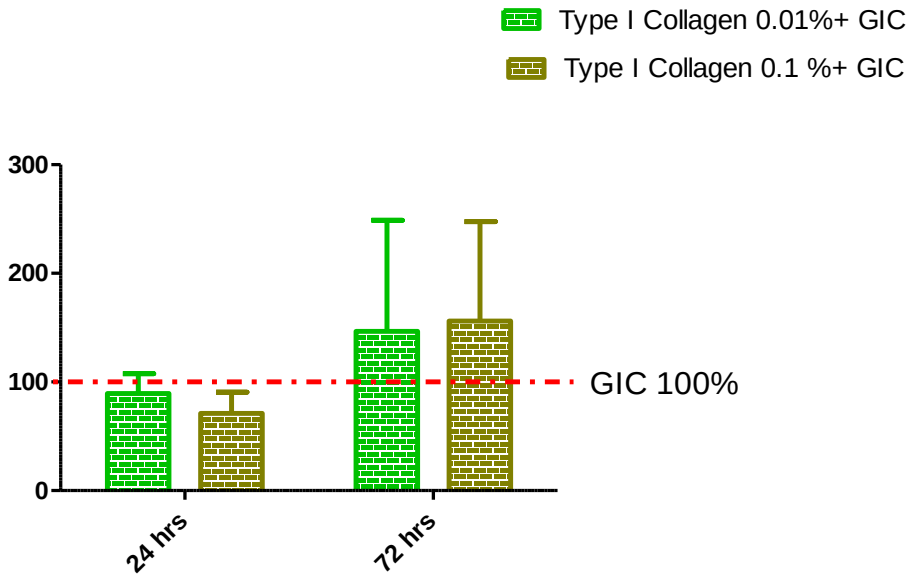

Figure 4-33 Percentage of cell viability (Mean+ SD) relative to the control (100% viability) for materials after 24 and 72 hours.

Statistical analysis (one-way ANOVA) showed significant effects between the groups of different Type I collagen concentration ($P<0.05$) on cell viability. Follow up

comparison by Tukey's multiple comparison test showed no significant differences between the different concentrations of the Type I collagen table (4-29).

Table 4-29 Tukey's comparison of means to different concentration of Type I collagen.

Versus	ChemFil Superior + 0.1% Type I collagen 24 hours	ChemFil Superior + 0.01% Type I collagen 72 hours
ChemFil Superior + 0.01% Type I collagen 24 hours	NS	___
ChemFil Superior + 0.01% Type I collagen 72 hours	___	NS

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

The effects of the addition of two different concentrations of RGD (1mg/ml, 5mg/ml) to ChemFil Superior on cell viability after 24 hours and 72 hours are presented in tables 4-30-31.

Table 4-30 Percentages of cell viability relative to the control (100% viability) after direct contact with the ChemFil superior modified with 1mg/ml and 5mg/ml RGD after 24 hours.

Sample	ChemFil Superior 24hrs	RGD 1mg/ml	RGD 5mg/ml
1	163.37	64.83	83.14
2	57.27	108.14	131.40
3	79.07	154.65	131.98
4	81.48	59.30	54.07
5	88.89	57.85	109.59
6	104.63	37.50	41.86
7	104.63	73.15	101.85
8	94.44	52.78	81.48
9	100.93	77.78	109.26
Mean	**97.19**	**76.22**	**93.85**
SD	29.05	35.33	31.56

Table 4-31 Percentages of cell viability relative to the control (100% viability) after direct contact with the ChemFil superior modified with 1mg/ml and 5mg/ml RGD after 72 hours.

Sample	ChemFil Superior 72hrs	RGD 1mg/ml	RGD 5mg/ml
1	107.78	73.56	294.00
2	94.44	94.22	48.44
3	98.00	95.78	229.78
4	93.33	81.33	176.44
5	102.22	112.89	224.22
6	102.22	83.78	93.33
7	90.24	115.56	84.44
8	100.00	135.56	128.89
9	107.32	88.89	106.67
Mean	**99.51**	**97.95**	**154.0**
SD	6.09	19.75	81.63

After 24 hours, cells exposed to bio- modified ChemFil Superior (1 mg/ml RGD and 5mg/ml RGD) showed less viability than the unmodified ChemFil Superior around 76.22% for 5mg/ml RGD + ChemFil superior and 93.85%5mg/ml RGD + ChemFil superior. After 72 hours the oral mucosa fibroblast cells associated with 5mg/ml RGD + ChemFil Superior showed greater viability then the cells associated with the control by more than 50% (Figure 4.34).

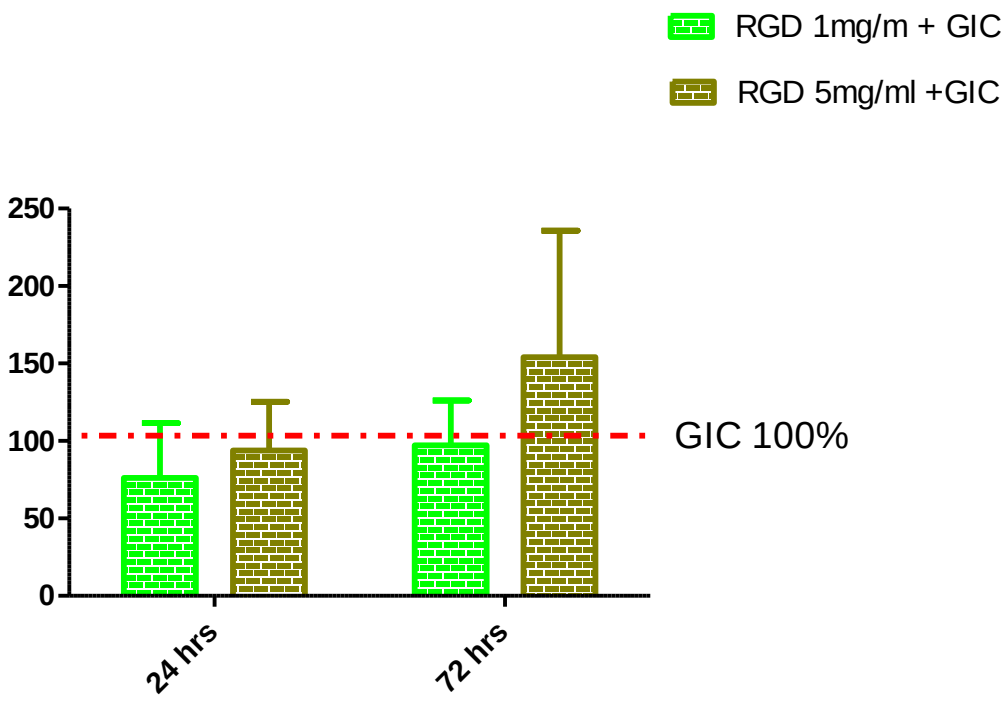

Figure 4-34 Percentage of cell viability (Mean+ SD) relative to the control (100% viability) for materials after 24 and 72 hours.

Statistical analysis (one-way ANOVA) showed highly significant effects between the groups of different RGD concentration ($P<0.001$) on cell viability. Follow up comparison by Tukey's multiple comparison test showed no significant differences between the different concentrations of the RGD (table 4-32).

Table 4-32 Tukey's comparison of means the different concentrations of the RGD of means.

Versus	ChemFil Superior + RGD 5mg/ml 24 hours	ChemFil Superior + RGD 5mg/ml 72 hours
ChemFil Superior + RGD 1mg/ml 24 hours	NS	—
ChemFil Superior + RGD 1mg/ml 72 hours	—	NS

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

The effects of the addition of two different concentrations of Emdogain (4mg/ml, 8mg/ml) to ChemFil Superior on cell viability after 24 hours and 72 hours are presented in tables 4-33 and 4-34.

Table 4-33 Percentages of cell viability relative to the control (100% viability) after direct contact with the ChemFil superior modified with 4mg/ml and 8mg/ml Emdogain after24 hours.

Sample	ChemFil Superior 24hrs	Emdogain 4mg/ml	Emdogain 8mg/ml
1	110.56	85.92	-1.41
2	77.46	48.59	-0.70
3	111.97	0.70	-1.41
4	69.29	15.49	21.13
5	113.28	9.15	-0.70
6	117.84	0.00	0.70
7	171.11	35.68	120.99
8	66.67	56.43	88.89
9	63.33	22.82	127.16
Mean	**100.2**	**30.53**	**39.41**
SD	34.84	28.84	56.11

Table 4-34 Percentages of cell viability relative to the control (100% viability) after direct contact with the ChemFil superior modified with 4mg/ml and 8mg/ml Emdogain after72 hours.

Sample	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml
1	95.37	33.70	108.93
2	121.82	64.89	106.90
3	82.66	119.44	81.03
4	68.00	173.67	168.50
5	144.00	133.39	40.60
6	88.00	122.57	112.85
7	110.48	99.15	140.00
8	70.82	135.98	120.00
9	118.98	59.49	196.00
Mean	**100..00**	**104.7**	**119.40**
SD	25.55	44.43	45.86

On the first 24 hours, cells exposed to bo A Kruskal-Wallis th bio- modified ChemFil Superior with 4 mg/ml Emdogain and 8mg/ml Emdogain showed very low viability compared with the control around 35. After 72 the viability of the cells associated with ChemFil superior modified with emdogain increased to excess the viability of the cells associated with unmodified ChemFil superior. (Figure 4-35).

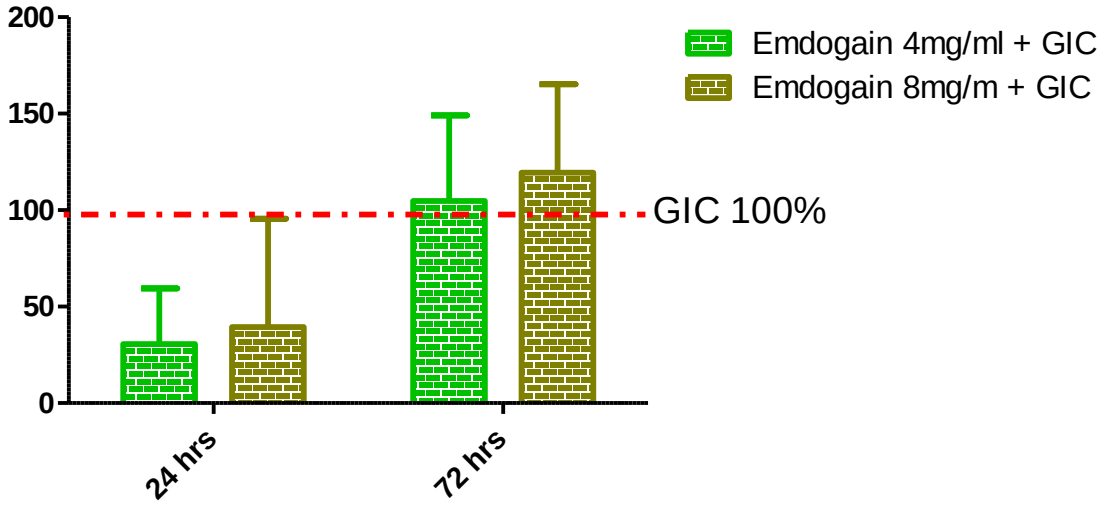

Figure 4-35 Percentage of cell viability (Mean+ SD) relative to the control (100% viability) for materials after 24 and 72 hours.

Statistical analysis (one-way ANOVA) showed very highly significant effects between the groups of different Emdogain concentration (*P<0.0001*) on cell viability. Follow up comparison by Tukey's multiple comparison test showed no significant differences between the different concentrations of Emdogain (table 4-35).

Table 4-35 Tukey's comparison of means to different concentrations of Emdogain of means.

Versus	ChemFil Superior + Emdogain 8mg/ml 24 hours	ChemFil Superior + Emdogain 8mg/ml 72 hours
ChemFil Superior + Emdogain 4mg/ml 24 hours	NS	___
ChemFil Superior + Emdogain 4mg/ml 72 hours	___	NS

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

4.2.1.2.3 DETERMINATION THE OPTIMAL BIOACTIVE ADDITIVES

To determine the optimal bio-additives to ChemFil superior glass ionomer and their effect on cell viability, MTT Assay was carried out after 24 hour and 72 hours of culturing oral mucosa fibroblast with bio-modified ChemFil Superior glass ionomer cement with 3 different bio- additives (0.1% type I Collagen, RGD 5 mg/ml, Emdogain 8mg/ml) which showed best cell viability. The absorbance values obtained for each well represent the amount of MTT reduction, which is proportional to the number of viable cells. In order to assess the percentage of viable cells present in each well, the absorbance values were related to those of the control (unmodified ChemFil Superior). This was achieved by setting the mean absorbance of the control at 100%.

The percentages of the effect of the bio-modifications of ChemFil Superior with different bio-additives (Collagen Type I 0.1%, RGD 5mg/ml, Emdogain 8mg/ml) on cell viability after 24 hours is presented in table 14 and the percentages of the effect of the Bio-modified ChemFil Superior on cell viability after 72 hours showed in tables 4-36 and 4-37.

Table 4-36 Percentages of cell viability relative to the control (100% viability) after direct contact with the ChemFil superior modified with RGD, Type I collagen and Emdogain after 24 hours.

Sample	ChemFil Superior 24hrs	RGD 5mg/ml	Type I Collagen 0.1%	Emdogain 8mg/ml
1	75.96	51.06	92.42	76.21
2	130.93	115.28	69.07	79.44
3	93.11	3.66	117.95	65.40
4	171.11	4.22	3.33	18.67
5	66.67	6.22	12.22	3.78
6	63.33	7.44	41.11	11.33
7	171.11	4.22	18.67	3.33
8	66.67	6.22	3.78	12.22
9	63.33	7.44	11.33	41.11
Mean	**100.2**	**49.54**	**65.25**	34.61
SD	45.56	44.87	40.20	31.54

Table 4-37 Percentages of cell viability relative to the control (100% viability) after direct contact with the ChemFil superior modified with RGD, Type I collagen and Emdogain after 72 hours.

Sample	ChemFil Superior 72hrs	RGD 5mg/ml	Type I Collagen 0.1%	Emdogain 8mg/ml
1	92.30	231.39	147.13	92.30
2	139.34	79.67	83.03	139.34
3	80.57	114.26	100.16	80.57
4	40.00	73.33	140.00	78.05
5	186.67	66.67	46.67	37.80
6	66.67	73.33	126.67	9.76
7	25.00	113.33	81.33	99.15
8	162.50	40.00	112.89	135.98
9	100.00	100.00	83.78	59.49
Mean	**99.23**	**98.67**	**124.6**	81.38
SD	54.47	40.15	76.99	42.27

On the first 24 hours (figure 1), cells exposed to ChemFil Superior modified with Collagen type I 0.1% showed highest viability around 65% where the cells exposed to ChemFil superior modified with RGD and Emdogain showed less viability with 49.54% and 34.61% respectively. After 72 hours the average percentages of the viability of the cells has been raised for all materials i.e. (ChemFil superior + collagen type I 124.60% , ChemFil superior + RGD 98.67 % , ChemFil superior + Emdogain 81.38 %) (Figure 4-36).

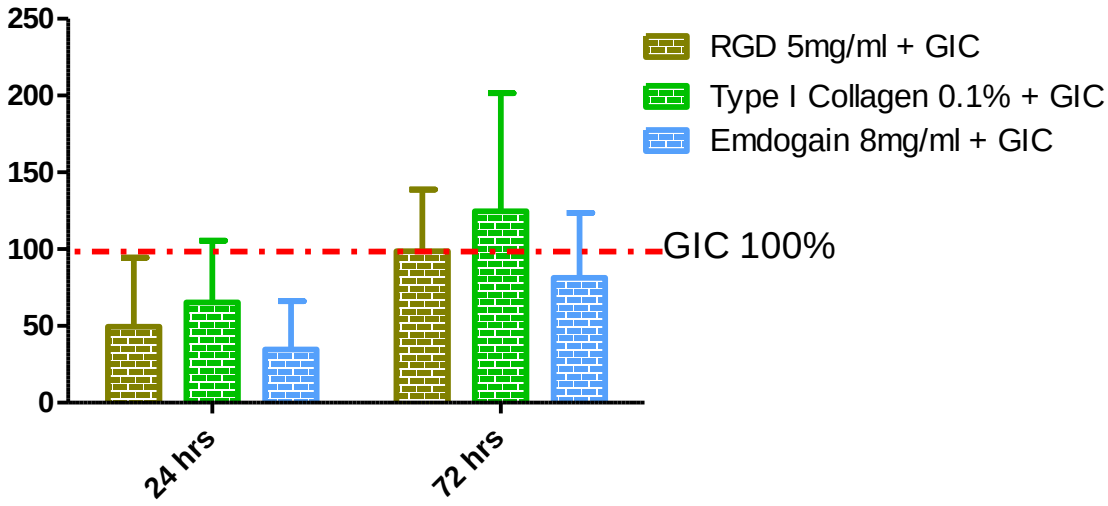

Figure 4-36 Percentage of cell viability (Mean+ SD) relative to the control (100% viability) for materials after 24 and 72 hours.

Statistical analysis (one-way ANOVA) showed very highly significant effects between the groups (*P<0.0001*) on cell viability. Follow up comparison by Tukey's multiple comparison test table 15 showed that no significant difference between cells viabilities for ChemFil superior modified with all different bio-additives after 24 hours. There was no significant deferent between all materials after 72 hours as well (Table 4-38).

Table 4-38 Tukey comparison of cell vibilty following different bio-addation .

Versus	0.1% Type I collagen 24 hours	0.1% Type I collagen 72 hours	RGD 5mg/ml 24 hours	RGD 5mg/ml 72 hours	Emdogain 8mg/ml 24 hours	Emdogain 8mg/ml 72 hours
0.1% Type I collagen 24 hours	___	___	NS	___	NS	___
0.1% Type I collagen 72 hours	___	___	___	NS	___	NS
RGD 8mg/ml 24 hours	NS	___	___	___	NS	___
RGD 5mg/ml 72 hours	___	NS	___	___	___	NS
Emdogain 8mg/ml 24 hours	NS	___	NS	___	___	___
Emdogain 8mg/ml 72 hours	___	NS	___	NS	___	___

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

4.2.1.3 *IMMUNOCYTOCHEMISTRY (ICC)*

After 21 days from culturing of oral mucosa fibroblast (MM1) cells with unmodified and bio-modified glass polyalkenoate cements (ChemFil Superior). The cells were incubated overnight with primary antibody against Vimentin and visualisation with a fluorescent secondary antibody.

In all cases the oral mucosa fibroblast cells expressed as revealed by immunofluorcsencence staining. Cells cultured around ChemFil superior + 0.1% type collagen showed highest level of Vimentin expression (Figure 4-37)

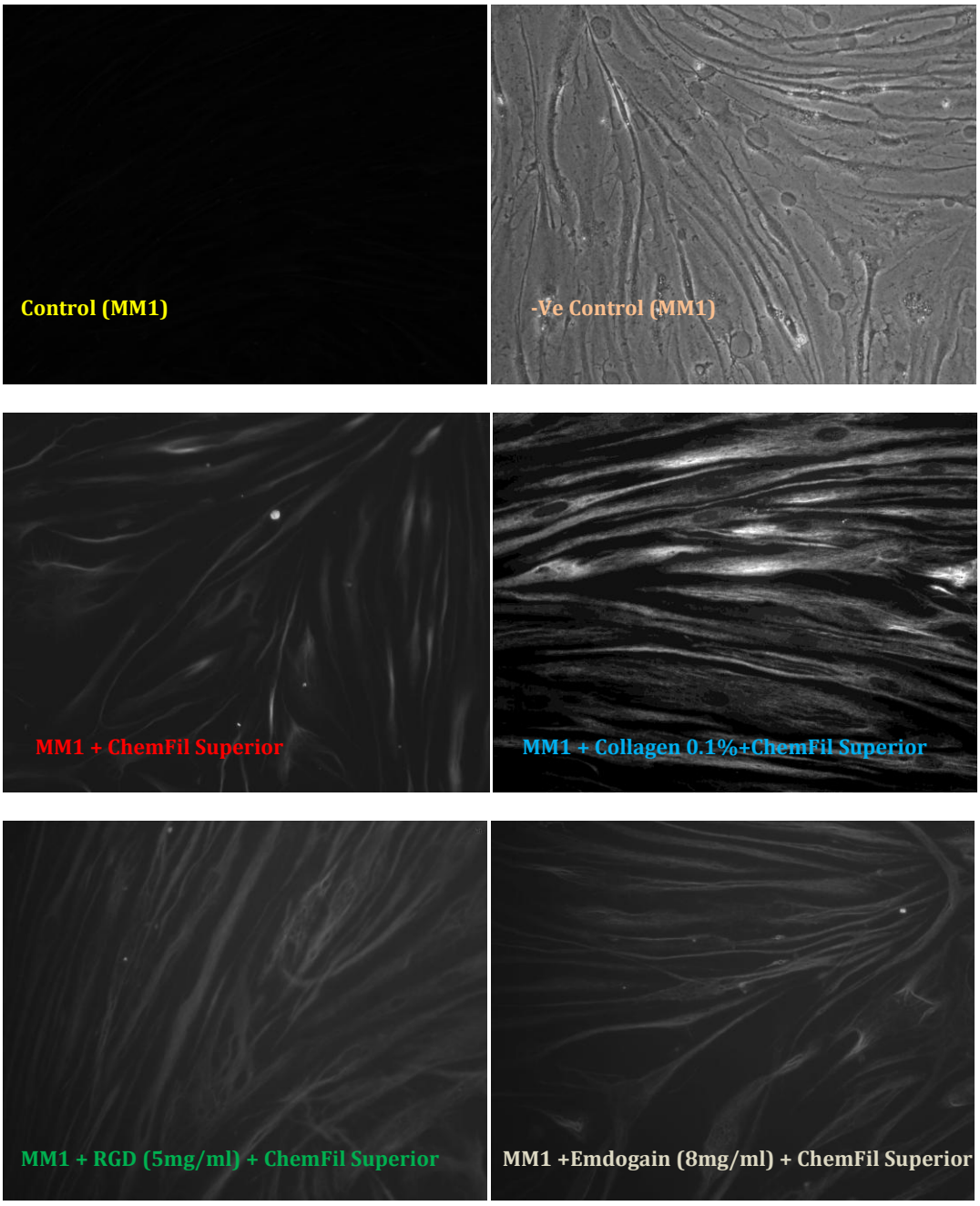

Figure 4-37 Oral mucosa fibroblast cultured in presence of the unmodified and bio-modified glass ionomer cement (ChemFil superior) and the same cultures fluorescently labelled for Vimentin. ChemFil superior + 0.1% type collagen showed highest level of Vimentin expres.

4.2.1.4 *PROTEIN BIOCHEMISTRY*

4.2.1.4.1 Western blotting

After 3 weeks of culturing MM1 with the materials, cells were lysed using RIPA buffer. Cell lysates were then run on DDS PAGE and then western blotted. The blots were then incubated with anti Vimentin antibody to see the differential expression of Vimentin by oral mucosal fibroblast cells when cultured with different materials. Cells treated with ChemFil Superior were used as a negative control. All cells used in this experiment expressed Vimentin but the level of expression varied depending upon the material present. Oral mucosa fibroblast cells cultured with modified ChemFil superior (GIC) with bio-additives specimens expressed more Vimentin compared to the cells cultured with unmodified ChemFil superior (GIC) alone protein expression was quantified from the Western blot using ImageLab software (BioRad 4.0.1. build 6) (Figure 4-38).

The highest percentage of Vimentin expression was associated with the cells cultured with ChemFil superior modified with collagen type I (58% more than the control GIC) (Figure 4-39)

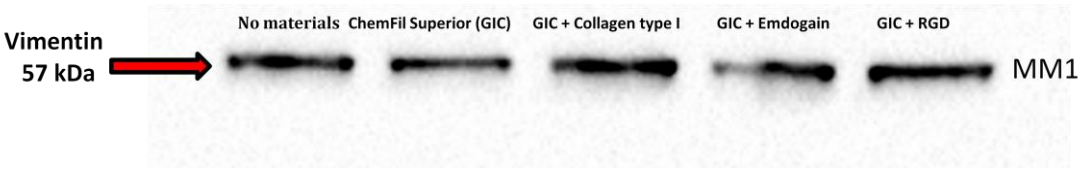

Figure 4-38 Vimentin expression from Oral mucosa fibroblast (MM1) cultured with Bio-modified and unmodified ChemFil superior (GIC).

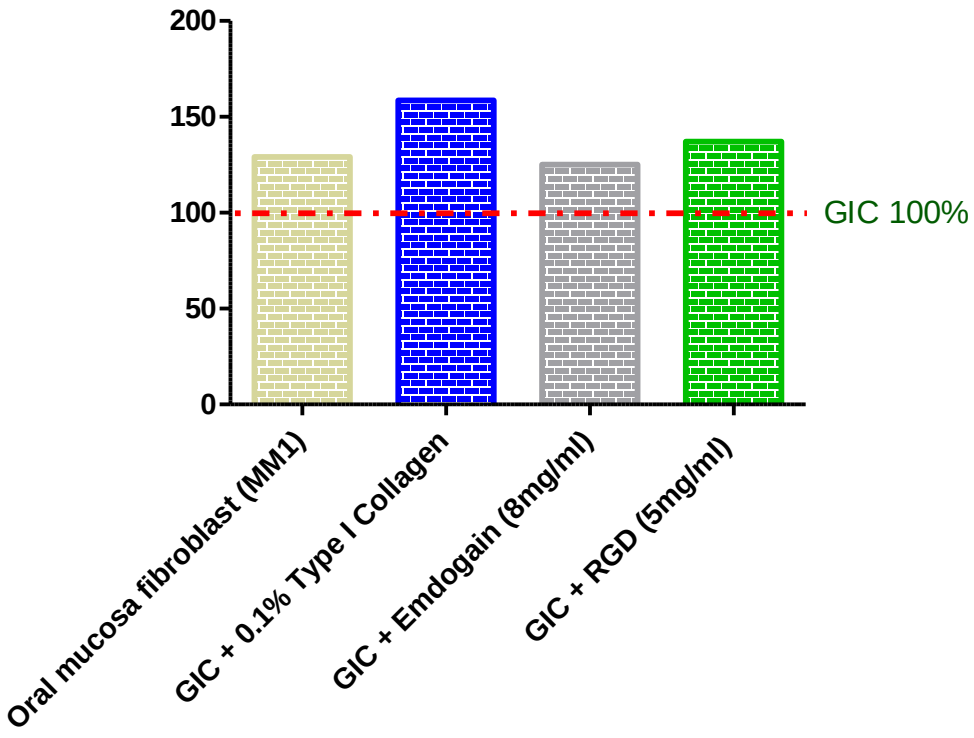

Figure 4-39 The percentage of Vimentin expressed by oral mucosa fibroblast cells (MM1) cultured with Bio-modified and unmodified ChemFil superior (GIC) where ChemFil superior represent 100%. Esmated using ImageLab softwear analysis of the western blot.

4.2.2 DETERMINATION OF THE PHYSICAL PROPERTIES OF THE GLASS POLYALKENOATE CEMENTS AT BASELINE AND FOLLOWING BIOACTIVE ADDITIONS RESULTS

The results of the physical properties are reported and analysed in this section in the same order as described in the materials and methods section

4.2.2.1 *SURFACE HARDNESS*

Table 4-39 gives the raw data from the testing of surface hardness of specimens of ChemFil Superior, GC Fuji VIII and ChemFil Superior with respective additions of RGD and Collagen type I. The units of measurement are shore hardness numbers. In additions the mean and standard deviation of these observations following one week of storage in distilled water are summarised in table 4-39 and Figure 4-40

Table 4-39 Raw data from the testing of surface hardness of specimens of ChemFil Superior, GC Fuji VIII and ChemFil Superior with respective additions of RGD and Type I Collagen.

Material	Raw shore Hardness values	Mean	Standard deviation
GC Fuji VII	85, 60, 70, 50, 50	63.0	14.8
ChemFil Superior	50, 30, 30,38, 38, 30	36.0	7.4
ChemFil Superior + RGD	38, 45, 65, 55,55,45	50.5	9.7
ChemFil Superior + Type I Collagen	45, 58, 55, 50, 50, 70	54.7	8.8

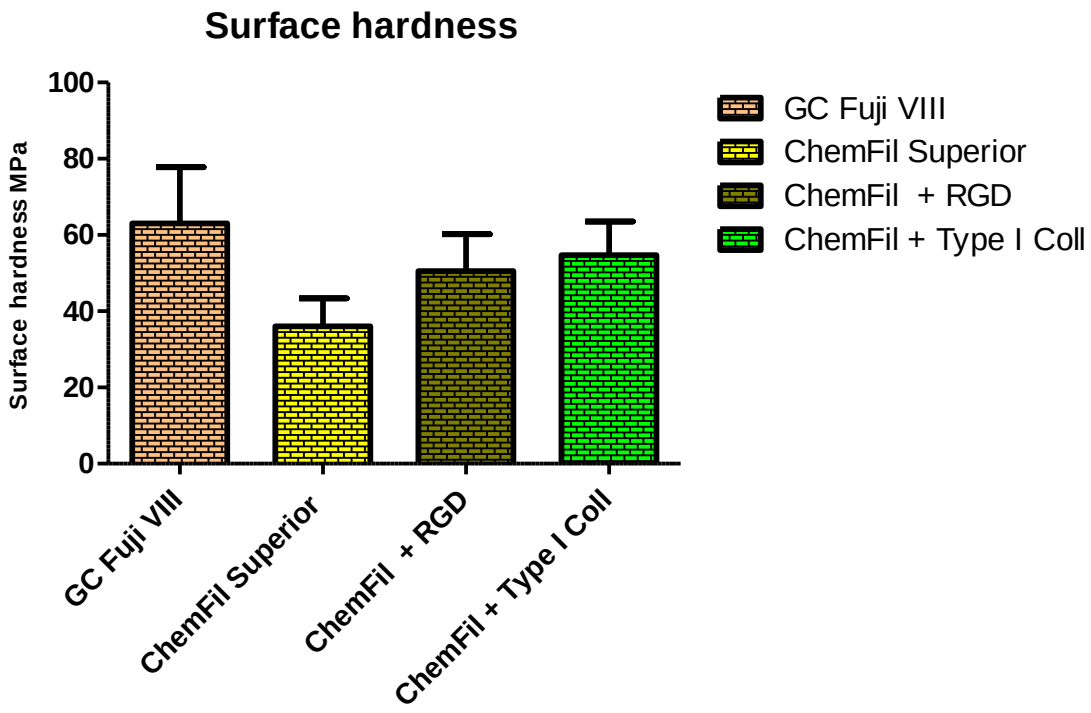

Figure 4-40 The mean and standard deviation of surface hardness of specimens of ChemFil Superior, GC Fuji VIII and ChemFil Superior with respective additions of RGD and Type I Collagen.

In order to compare these results a normality test was performed. This confirmed that the data followed a normal statistical distribution. Accordingly a one way analysis of variance was performed. This highlighted significant differences (P = 0.0029) in these values. These were localised using a Tukey comparison of means as summarised in table 4-40.

Table 4-40 Tukey comparison of means of hardness values.

Versus	ChemFil Superior	ChemFil Superior + RGD	ChemFil Superior + Collagen I
GC Fuji VIII	**	NS	NS
ChemFil Superior	___	NS	*
ChemFil Superior + RGD		—	NS

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

The Shore hardness of ChemFil Superior was significantly lower than that for GC Fuji VIII. Addition of RGD and Collagen I to ChemFil Superior did not impair its surface hardness and in the case of collagen I addition significantly ($P < 0.05$) increased it.

4.2.2.2 *COMPRESSIVE STRENGTH*

Tables 4-41 -4-44 give the dimensions (length, diameter) and force at failure for compressive specimens together with their calculated compressive strength.

Table 4-41 Compressive strength of GC Fuji VIII measured at 1mm/ min following one week of storage in distilled water.

Specimen number	Length (mm)	Diameter (mm)	Failure (N)	Compressive strength (MPa)
1	6.39	4.19	314.1	22.8
2	6.53	4.12	649.7	48.8
3	6.32	4.27	982.5	68.6
4	6.43	4.12	790.6	59.3
5	6.40	4.27	353.0	24.7
6	6.35	4.32	687.2	46.9
7	6.31	4.26	1548.0	108.7
8	6.37	4.20	479.2	34.6
9	6.49	4.18	1554.0	113.3
10	6.63	4.28	1232.0	85.7
11	6.57	4.16	864.4	63.6
12	6.38	4.19	661.7	48.0
13	6.39	4.30	1109.0	76.4
14	6.27	4.18	1624.0	118.4
15	6.24	4.10	582.6	44.2
16	6.39	4.37	1370.0	91.4

Table 4-42 Compressive strength of GC ChemFil Superior measured at a across head speed 1mm /min following one week of storage in distilled water.

Specimen number	Length (mm)	Diameter(mm)	Failure (N)	Compressive strength (MPa)
1	6.37	4.21	1009.1	72.5
2	6.59	4.12	494.0	48.8
3	6.81	4.30	661.7	45.6
4	6.45	4.10	413.4	31.3
5	6.55	4.10	547.7	41.5
6	6.60	4.01	472.5	37.4
7	6.31	4.01	843.0	66.8
8	6.34	4.14	795.7	35.6
9	6.40	4.21	1350.0	97.0
10	6.49	4.10	880.0	66.7
11	6.38	4.12	573.2	43.0
12	6.45	4.24	785.2	55.6
13	6.51	4.14	706.0	50.0
14	6.28	4.16	447.0	33.2
15	6.43	4.11	307.4	22.6

Table 4-43 Compressive strength of GC ChemFil Superior + RGD measured at 1mm/ min following one week of storage in distilled water.

Specimen number	Length (mm)	Diameter (mm)	Failure (N)	Compressive strength (MPa)
1	6.30	4.13	1624.0	121.3
2	6.28	4.18	1722.0	125.6
3	6.32	4.10	910.1	69.0
4	6.46	4.30	1392.0	95.9
5	6.16	4.19	1119.0	81.2
6	6.14	4.22	325.0	23.3
7	6.35	4.16	1536.0	113.1
8	6.16	4.90	1419.0	75.3
9	6.46	4.24	1427.0	101.1
10	6.33	4.18	1242.0	90.6
11	6.26	4.22	869.8	62.2
12	6.27	4.16	1744.0	128.4
13	6.22	4.16	1858.0	136.8
14	6.34	4.20	1070.0	77.3
15	6.22	4.26	1644.0	115.4
16	6.39	4.60	1107.0	66.6

Table 4-44 Compressive strength of GC ChemFil Superior + Collagen I measured at a across head speed 1mm/ min following one week of storage in distilled water.

Specimen number	Length (mm)	Diameter(mm)	Failure (N)	Compressive strength (MPa)
1	6.17	4.11	1850.0	139.5
2	6.53	4.25	1003.0	70.7
3	6.28	4.22	1512.0	108.2
4	6.46	4.26	1281.0	89.9
5	6.31	4.32	1713.0	116.9
6	6.51	4.12	526.2	39.5
7	6.34	4.11	1592.0	120.1
8	6.24	4.70	1415.0	81.6
9	6.38	4.18	1298.0	94.6
10	6.45	4.24	1758.0	124.6
11	6.90	4.18	1283.0	93.5
12	6.28	4.19	1436.0	104.2
13	6.44	4.11	1742.0	131.4
14	6.12	4.14	1420.0	105.5
15	6.26	4.50	1711.0	89.3

The mean and standard deviations of the all compressive strengths are summarised in Table 4-45 and Figure 4-41.

Table 4-45 The mean and standard deviations of all compressive strengths determined.

Properties/Material	Compressive strength (MPa)
GC Fuji $VIII_{GP}$	66.0 (31.0) n = 16
ChemFil Superior	50.0 (19.0) n = 15
ChemFil Superior + RGD	93.0 (30.0) n = 15
ChemFil Superior + Collagen I	101.0 (25.0) n = 15

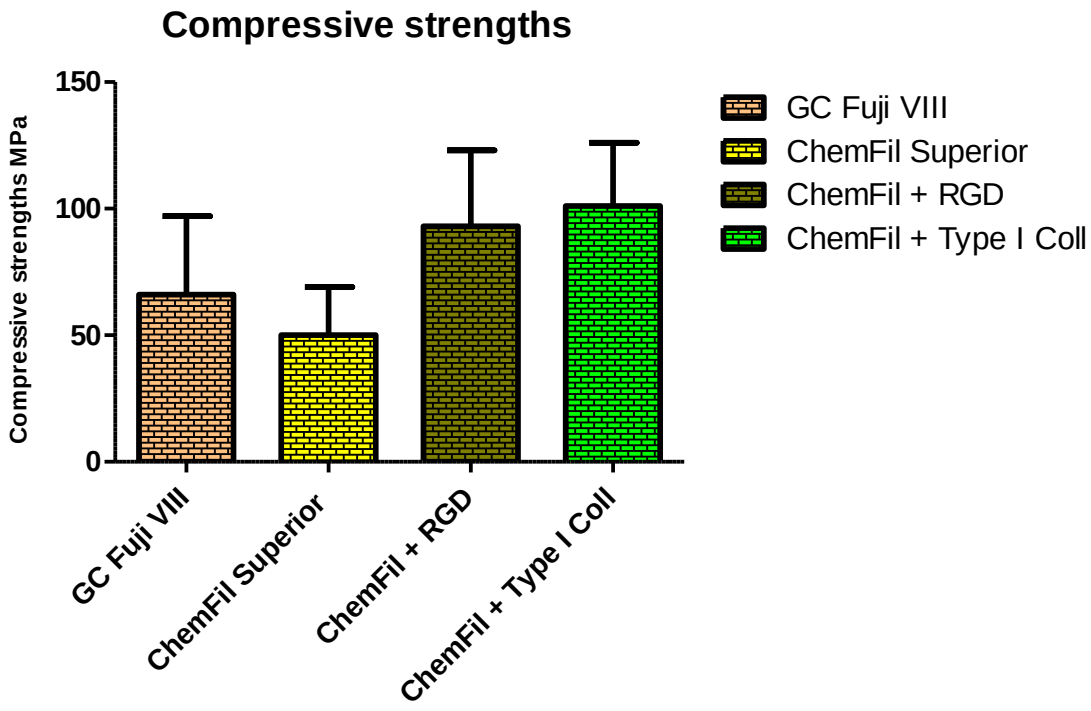

Figure 4-41 The mean and standard deviations of all compressive strengths determined.

The data conformed to a normal statistical distribution. Accordingly an analysis of variance was conducted and revealed highly statistically significant difference ($P < 0.0001$) between these values. These were localised using a Tukey comparison of means whose outcome is summarised in table 4-46. This demonstrated that the addition of RGD and collagen I significantly increased compressive strength.

Table 4-46 Tukey comparison of means of compressive strength.

Versus	ChemFil Superior + RGD	ChemFil Superior + Collagen I
ChemFil Superior	***	***
ChemFil Superior + RGD	—	NS

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

4.2.2.3 *DIAMETRAL COMPRESSIVE STRENGTH*

Tables 4-47-4-50 give the dimensions (length, diameter) and force at failure for all diametral compressive specimens together with their calculated diametral compressive strength.

Table 4-47 Diametral strength of GC Fuji VIII following one week of storage in distilled water.

Specimen number	Length (mm)	Diameter (mm)	Failure (N)	Diametral strength (MPa)
1	6.42	4.34	635.7	14.5
2	6.41	4.18	547.7	13.0
3	6.34	4.09	527.5	12.9
4	6.28	4.21	947.6	22.8
5	6.48	4.15	461.7	10.9
6	6.54	4.21	549.0	12.7
7	6.45	4.13	404.0	9.7
8	6.36	4.18	426.9	10.2
9	6.22	4.40	554.4	13.0
10	6.47	4.10	546.3	13.1
11	6.50	4.16	614.8	14.5
12	6.48	4.13	562.4	13.4
13	6.41	4.14	540.9	13.0
14	6.37	4.17	680.5	16.3
15	6.51	4.17	590.6	13.9

Table 4-48 Diametral strength of GC ChemFil following one week of storage in distilled water.

Specimen number	Length (mm)	Diameter (mm)	Failure (N)	Diametral strength (MPa)
1	6.18	3.95	255.0	6.7
2	6.18	4.15	259.1	6.4
3	6.65	4.20	331.5	7.6
4	6.16	4.15	257.7	6.4
5	6.48	4.12	340.9	8.1
6	5.97	4.23	306.0	7.7
7	6.45	4.34	311.4	7.1
8	6.38	4.01	230.9	5.8
9	6.24	4.13	236.2	5.8
10	6.29	4.07	322.2	8.0
11	6.07	4.27	145.0	3.6
12	6.21	4.11	204.0	5.1
13	6.19	3.97	169.1	4.4
14	6.40	4.05	489.9	12.0
15	6.18	4.12	166.5	4.2

Table 4-49 Diametral strength of GC ChemFil + RGD following one week of storage in distilled water.

Specimen number	Length (mm)	Diameter (mm)	Failure (N)	Diametral strength (MPa)
1	6.21	4.25	444.3	10.7
2	6.29	4.09	378.5	9.4
3	6.03	4.16	410.7	10.4
4	6.24	4.21	578.5	14.0
5	6.14	4.08	366.4	9.3
6	5.42	4.09	392.0	11.5
7	6.31	4.14	451.0	11.0
8	6.36	4.10	539.6	13.2
9	6.35	4.10	382.6	9.4
10	6.09	4.16	401.3	10.1
11	6.46	4.29	355.7	8.2
12	6.34	4.22	453.7	10.8
13	6.33	3.09	532.9	17.4
14	6.21	4.26	261.8	6.3
15	6.35	4.23	577.2	13.7

Table 4-50 Diametral strength of GC ChemFil + Collagen I following one week of storage in distilled water.

Specimen number	Length (mm)	Diameter (mm)	Failure (N)	Diametral strength (MPa)
1	6.47	4.35	550.3	12.5
2	6.12	4.19	477.9	11.9
3	6.15	4.14	374.5	9.4
4	6.44	4.18	232.2	5.5
5	6.44	4.08	487.3	11.8
6	6.27	4.09	461.7	11.5
7	6.27	4.16	471.1	11.5
8	6.43	4.26	511.4	11.9
9	6.08	4.27	475.2	11.7
10	6.18	4.29	437.6	10.5
11	6.14	4.20	483.2	11.9
12	6.46	4.30	469.8	10.8
13	6.10	4.32	351.7	8.5
14	6.13	4.16	218.8	5.5
15	6.33	4.19	373.2	9.0

The mean and standard deviations of the diametral compressive strength testing are summarised in table 4-51 and figure 4-42

Table 4-51 The mean and standard deviations of all the diametral compressive strength determined

Properties/Material	Diametral compressive strength (MPa)
GC Fuji VIII$_{GP}$	13.6 (3.1) n = 15
ChemFil Superior	6.6 (2.1) n = 15
ChemFil Superior + RGD	11.0 (2.7) n = 15
ChemFil Superior + Collagen I	10.2 (2.3) n = 15

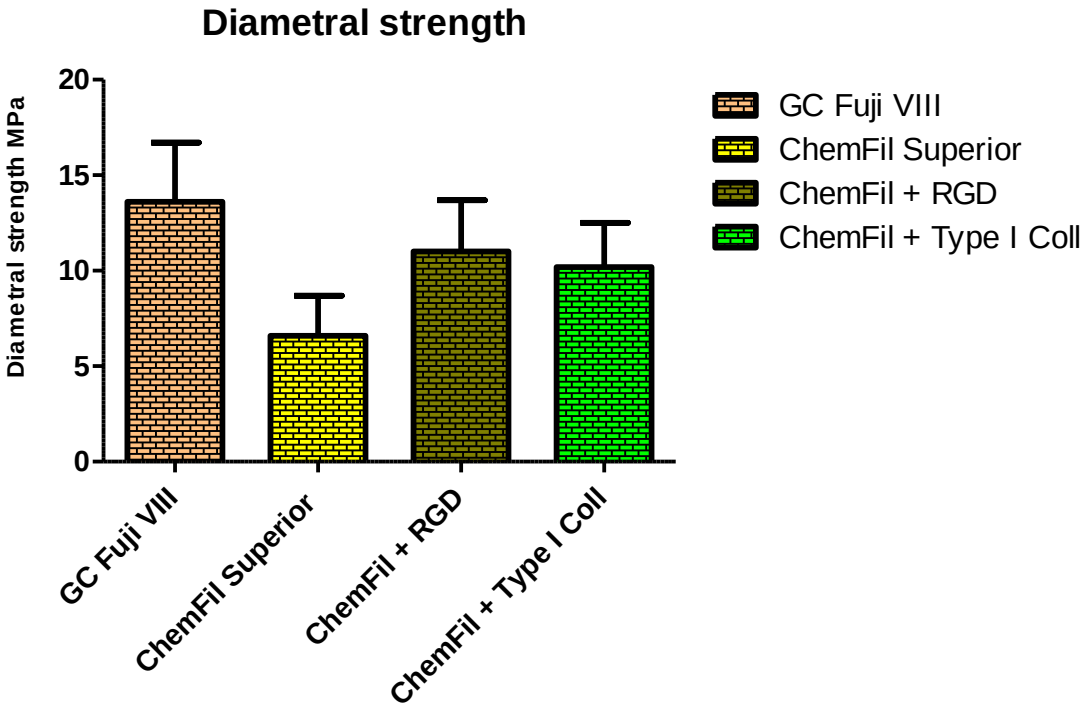

Figure 4-42 The mean and standard deviations of all the diametral compressive strength determined.

The data conformed to a normal statistical distribution. Accordingly an analysis of variance revealed highly statistically significant differences ($P < 0.0001$) between these values. These were localised using a Tuky comparison of means whose outcome in summary in table 4-52 this demonstrates that the addition of RGD and collagen I significantly increased diametral compressive strength

Table 4-52 Tukey comparison of means to diametral compression of means.

Versus	ChemFil Superior + RGD	ChemFil Superior + Collagen I
ChemFil Superior	***	***
ChemFil Superior + RGD	—	NS

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

4.2.2.4 *3 POIN FLEXURAL STRENGTH*

Tables 4-53 -4-56 gives the dimensions (Depth, Width) and force at failure for all flexural strength specimens their together with calculated 3 point flexural strength.

Table 4-53 3 point flexural strength of GC Fuji VIII measured at a across head speed 1mm/ min following one week of storage in distilled water.

Specimen number	Depth (mm)	Width (mm)	Failure (N)	3 points flexural strength (MPa)
1	2.50	3.02	7.0	11.1
2	2.52	3.16	7.0	10.5
3	2.59	3.25	7.0	9.6
4	2.56	3.36	10.0	13.6
5	2.54	3.29	0.0	0
6	2.54	3.35	7.0	9.7
7	2.58	3.40	10.0	13.3
8	2.53	3.13	10.0	15.0
9	2.55	3.20	10.0	14.4
10	2.57	3.09	10.0	14.7
11	2.64	3.29	10.0	13.1
12	2.54	3.21	17.0	24.6
13	2.66	3.29	12.0	15.5
14	2.78	3.22	10.0	12.1
15	2.76	3.12	12.0	15.2
16	2.65	3.28	12.0	11.1

Table 4-54 3 point flexural strength of GC ChemFil Superior measured at a across head speed 1mm/ min following one week of storage in distilled water.

Specimen number	Depth (mm)	Width (mm)	Failure (N)	3 points flexural strength (MPa)
1	2.51	3.22	5.4	8.0
2	2.37	3.11	6.7	11.5
3	2.43	3.12	6.7	10.9
4	2.79	3.33	8.1	9.4
5	2.46	3.18	12.1	18.9
6	2.58	3.24	9.4	13.1
7	2.60	3.29	14.8	20.0
8	2.48	3.25	6.7	10.1
9	2.50	3.33	8.1	11.7
10	2.51	3.14	10.7	16.2
11	2.54	3.31	5.4	7.6
12	2.63	3.16	5.4	7.4
13	2.67	3.23	8.1	10.6
14	2.53	3.21	6.7	9.8
15	2.45	3.25	6.7	10.3
16	2.56	3.19	8.1	8.0

Table 4-55 3 point flexural strength of GC ChemFil Superior measured at a across head speed 1mm/ min following one week of storage in distilled water.

Specimen number	Depth (mm)	Width (mm)	Failure (N)	3 points flexural strength (MPa)
1	2.56	3.32	4.0	5.5
2	2.35	3.21	12.1	20.5
3	2.72	3.33	20.1	24.5
4	2.51	3.60	12.1	16.0
5	2.46	3.63	16.1	22.0
6	2.60	3.22	4.0	5.5
7	2.55	3.28	6.7	9.4
8	2.43	3.05	5.4	9.0
9	2.34	3.14	5.4	9.4
10	2.47	3.20	4.0	6.2
11	2.38	3.03	4.0	7.0
12	2.59	3.25	4.0	5.5
13	2.41	3.31	5.4	8.4
14	2.54	3.26	4.0	5.7
15	2.40	3.26	6.7	10.7

Table 4-56 3 point Flexural strength of GC ChemFil + RGD measured at a across head speed 1mm min following one week of storage in distilled water.

Specimen number	Depth (mm)	Width (mm)	Failure (N)	3 points flexural strength (MPa)
1	2.40	3.08	9.4	15.9
2	2.50	3.14	17.5	26.8
3	2.55	3.11	6.7	9.9
4	2.40	3.11	14.8	24.8
5	2.60	3.05	8.1	11.8
6	2.55	3.04	6.7	10.2
7	2.57	3.18	9.4	13.4
8	2.49	3.22	10.7	16.1
9	2.43	3.19	6.7	10.7
10	2.51	3.08	13.4	20.7
11	2.36	3.09	12.1	21.1
12	2.53	3.11	10.7	16.1
13	2.38	3.04	8.1	14.1
14	2.49	3.22	16.1	24.2
15	2.47	3.04	6.7	10.8

The mean and standard deviations of the 3 points flexural strengths are summarised in table 4-57 and figure 4-43

Table 4-57 The mean and standard deviations of all the 3 points Flexural strengths.

Properties/Material	3 points flexural strength (MPa)
GC Fuji VIII$_{GP}$	12.7 (3.1) n = 16
ChemFil Superior	11.4 (3.8) n =16
ChemFil Superior + RGD	16.4 (5.7) n = 15
ChemFil Superior + Collagen I	12.6 (2.8) n = 15

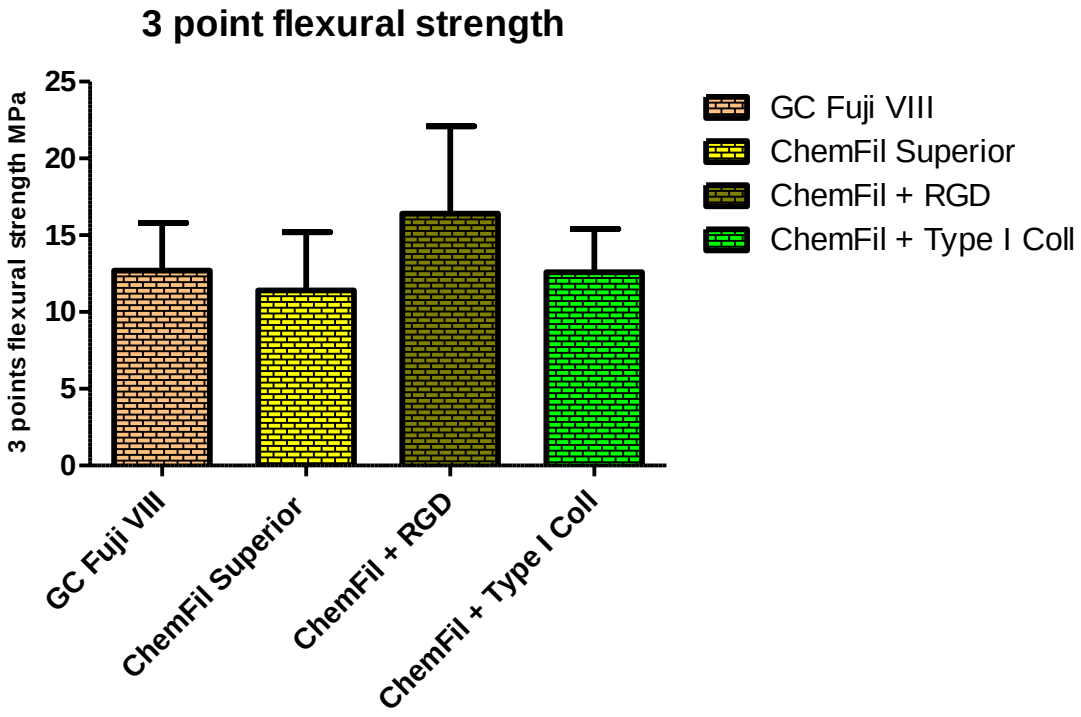

Figure 4-43 The mean and standard deviations of all the 3 point Flexural strengths.

The data conformed to a normal statistical distribution. Accordingly an analysis of variance revealed highly statistically significant differences ($P < 0.0063$) between these values. These were localised using a Tukey comparison of means whose outcome in summary in table 4.58 this demonstrates that the addition of RGD significantly increased 3 points Flexural strength

Table 4-58 Tukey's comparison of means to 3 point flexural strengths .

versus	ChemFil Superior + RGD	ChemFil Superior + Collagen I
ChemFil Superior	**	NS
ChemFil Superior + RGD	—	*

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

4.2.2.5 *DIAMETRAL COMPRESSIVE FATIGUE LIMIT*

Tables 4-59 to 4-62 give the applied load (N) and number of cycles to survival (at 1500 cycles or above) or failure for the glass ionomer ChemFil Superior and GC Fuji VIII following one week storage in distilled water at 37°C. The results are also given for the additions of RGD and Collagen I to ChemFil Superior.

Table 4-59 Diametral fatigue of GC ChemFil following one week of storage in distilled water.

Specimen number	Applied load (N)	Number of cycles	Survived(S)/Failed(F)	Applied stress (MPa)
1	100	1500	S	2.65
2	110	320	F	2.92
3	100	1500	S	2.65
4	110	77	F	2.92
5	100	1500	S	2.65
6	110	661	F	2.92
7	100	486	F	2.65
8	90	136	F	2.39
9	80	1500	S	2.12
10	90	1238	F	2.39
11	80	282	F	2.12
12	70	512	F	1.86
13	60	1500	S	1.59
14	70	1500	S	1.86
15	80	1500	S	2.12

Table 4-60 Diametral fatigue of GC Fuji VIII following one week of storage in distilled water.

Specimen number	Applied load (N)	Number of cycles	Survived(S)/Failed(F)	Applied stress (MPa)
1	344	792	F	9.13
2	334	444	F	8.86
3	324	817	F	8.60
4	314	191	F	8.33
5	304	795	F	8.07
6	294	130	F	7.80
7	284	756	F	7.54
8	274	380	F	7.27
9	264	311	F	7.01
10	254	831	F	6.74
11	244	1500	S	6.48
12	254	1500	S	6.74
13	264	1500	S	7.01
14	274	1500	S	7.27
15	284	1354	F	7.34

Table 4-61 Diametral fatigue of GC ChemFil + RGD following one week of storage in distilled water.

Specimen number	Applied load (N)	Number of cycles	Survived(S)/Failed(F)	Applied stress (MPa)
1	240	776	F	6.4
2	230	1500	S	6.1
3	240	273	F	6.4
4	230	1500	S	6.1
5	240	1500	S	6.4
6	250	750	F	6.6
7	240	730	F	6.4
8	230	1500	S	6.1
9	240	1500	S	6.4
10	250	1500	S	6.6
11	260	695	F	6.9
12	250	220	F	6.6
13	240	250	F	6.4
14	230	1500	S	6.1
15	240	550	F	6.4

Table 4-62 Diametral Fatigue of GC ChemFil + Collagen I following one week of storage in distilled water.

Specimen number	Applied load (N)	Number of cycles	Survived(S)/Failed(F)	Applied stress (MPa)
1	100	1500	S	2.7
2	110	1500	S	2.9
3	120	1500	S	3.2
4	130	1500	S	3.5
5	140	1500	S	3.7
6	150	1500	S	4.0
7	160	1500	S	4.3
8	170	1500	S	4.5
9	180	850	F	4.8
10	170	1500	S	4.5
11	180	920	F	4.8
12	170	1500	S	4.5
13	180	1253	F	4.8
14	170	1500	S	4.5
15	180	1374	F	4.8

Table 4-63 summarises the calculated fatigue limits for these results and their standard deviations. Comparison of these values by a student's t test is summarised in table 4-64 and figure 4-44.

Table 4-63 The calculated diametral fatigue limits and their standard deviations for the glass ionomer cements. The fatigue limit is the product of testing 15 specimens.

Material and additions	Fatigue limit (MPa)	Standard Deviation
GC Fuji $VIII_{GP}$	6.9	0.1
ChemFil Superior	2.2	1.2
ChemFil Superior + RGD	6.9	0.6
ChemFil Superior + Collagen I	4.7	0.1

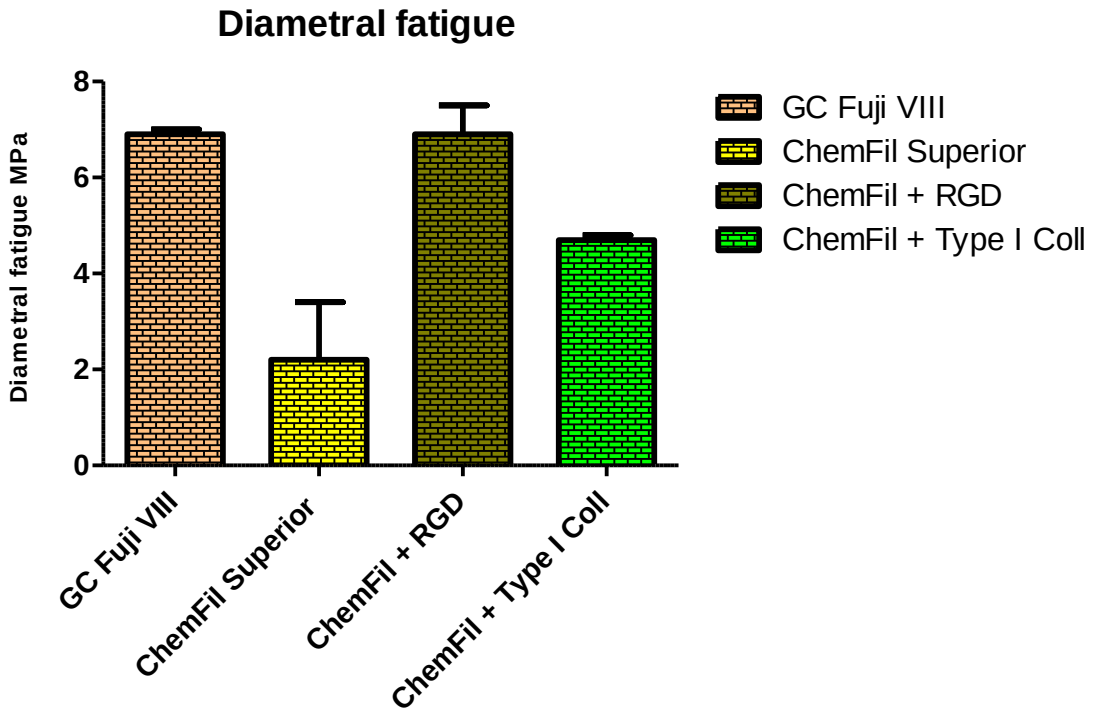

Figure 4-44 The calculated diametral fatigue limits and their standard deviations for the glass ionomer cements.

Table 4-64 Summary of student's t test of diametral compressive fatigue specimens.

versus	ChemFil Superior	ChemFil Superior + RGD	ChemFil Superior + Collagen I
GC Fuji VIII	***	NS	***
ChemFil Superior	___	***	***
ChemFil Superior + RGD		___	***

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

It is clear that GC Fuji VIII exhibits a superior fatigue limit than ChemFil Superior ($P < 0.001$) but addition of RGD to ChemFil Superior increases the value of this property to level where it does not differ significantly ($P > 0.05$) From Fuji VIII.

Additions of both RGD and collagen I to ChemFil Superior significantly ($p < 0.001$) improve its diametral fatigue limit but this improvement is significantly greater ($P < 0.001$) for RGD than that achieved by collagen I addition.

4.2.2.6 *BIAXIAL FLEXURAL STRENGTH*

Tables 4-65-4.68 give the thickness and force at failure of biaxial flexural strength specimens together with the associated calculated biaxial flexural strength.

Table 4-65 Biaxial flexural strength of GC Fuji VIII following one week of storage in distilled water.

Specimen number	Thickness (mm)	Applied load (N)	Biaxial flexural strength (MPa)
1	2.10	179.9	80.0
2	1.91	182.9	81.3
3	2.22	196.0	87.2
4	2.08	157.1	69.9
5	2.23	202.1	89.9
6	1.97	112.1	49.8
7	1.98	159..7	71.0
8	1.88	159.7	71.0
9	2.08	170.5	75.8
10	1.95	138.3	61.5

Table 4-66 Biaxial flexural strength of GC ChemFil following one week of storage in distilled water.

Specimen number	Thickness (mm)	Applied load (N)	Biaxial flexural strength (MPa)
1	2.07	37.6	16.7
2	1.91	38.9	17.3
3	2.04	29.5	13.1
4	1.91	83.2	37.0
5	2.23	59.1	26.3
6	1.85	49.7	22.1
7	2.01	44.3	19.7
8	2.07	60.4	26.9
9	1.92	36.3	16.1
10	1.85	51.0	22.7

Table 4-67 Biaxial flexural strength of GC ChemFil + RGD following one week of storage in distilled water.

Specimen number	Thickness (mm)	Applied load (N)	Biaxial flexural strength (MPa)
1	1.83	53.7	23.9
2	1.87	59.9	26.6
3	1.85	68.5	30.5
4	2.05	80.5	35.8
5	1.89	61.8	27.5
6	1.80	63.1	28.1
7	1.94	67.1	29.8
8	1.60	83.1	37.0
9	1.94	89.9	40.0
10	1.93	85.9	38.2

Table 4-68 Biaxial flexural strength of GC ChemFil + Collagen I following one week of storage in distilled water.

Specimen number	Thickness (mm)	Applied load (N)	Biaxial flexural strength (MPa)
1	1.96	108.7	48.3
2	1.88	75.5	33.6
3	1.94	64.4	28.6
4	2.02	79.2	35.2
5	1.83	72.5	32.2
6	1.93	77.9	34.6
7	1.90	73.8	32.8
8	1.96	67.1	29.8
9	1.99	80.5	35.8
10	2.00	84.6	37.6

The mean and standard deviations of the biaxial flexural strength testing are and summarised in table 4.69 and figure 4-45.

Table 4-69 The mean and standard deviation of the Biaxial flexural strengths of the glass ionomer cements.

Properties/Material	Biaxial flexural strength (MPa)
GC Fuji VIII$_{GP}$	73.7 (12.0) n = 10
ChemFil Superior	21.8 (7.0) n =10
ChemFil Superior + RGD	31.7 (5.6) n = 10
ChemFil Superior + Collagen I	34.9 (5.5) n = 10

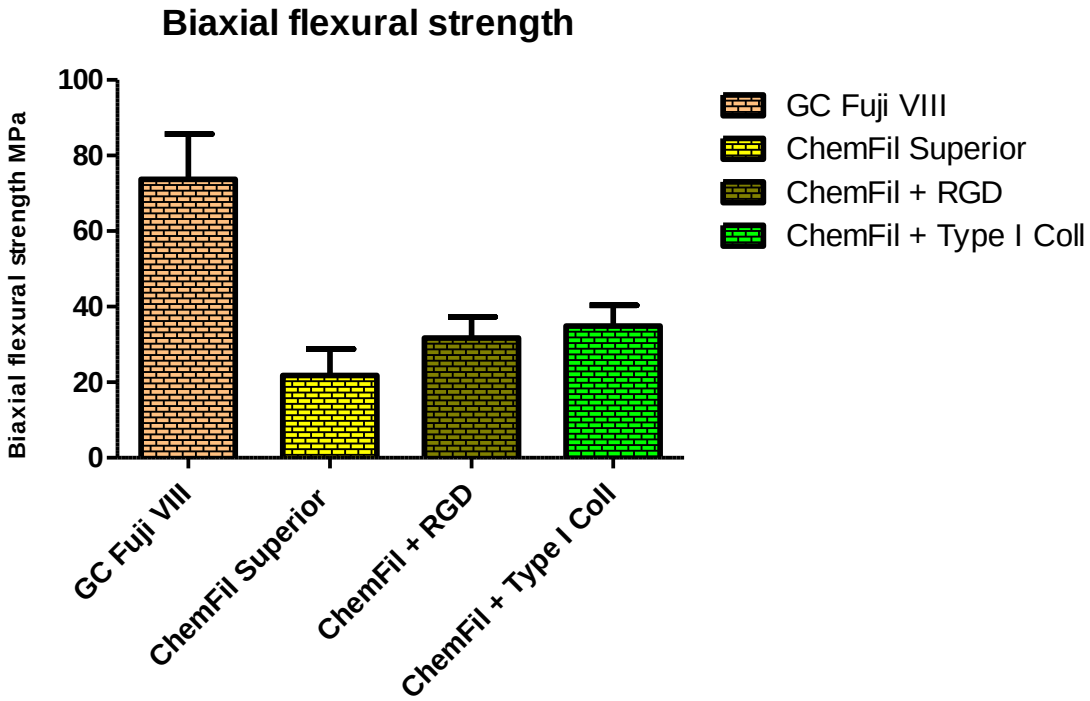

Figure 4-45 The mean and standard deviation of the Biaxial flexural strengths of the glass ionomer cements.

An analysis of variance revealed highly statistically significant difference ($P < 0.0001$) between these is values. These were localised using a Tuky compression of means whose outcome summarised in table 4-70. This demonstrates that the addition of RGD and collagen I significantly increased diametral Biaxial flexural strength.

Table 4-70 Tukey comparison of means to biaxial flexural strengths.

Versus	ChemFil Superior + RGD	ChemFil Superior + Collagen I
ChemFil Superior	**	***
ChemFil Superior + RGD	—	NS

Key; NS = not significant, * = p < 0.05, **= p < 0.01, *** = p < 0.0001

4.2.2.7 *ADHESIVE SHEAR BOND STRENGTHS*

Tables 4.71-4.74 give the force at failure for Adhesive shear bond strength specimens together with the associated calculated adhesive shear bond strengths.

Table 4-71 Adhesive shear bond strength of GC Fuji VIII following one week of storage in distilled water.

Specimen number	Applied load (N)	Bond shear strength
1	22.8	1.9
2	41.6	3.5
3	0.0	0
4	55.0	4.7
5	4.0	0.3
6	0	0
7	103.4	8.8
8	218.8	18.6
9	5.0	0.4
10	32.2	2.7
11	0	0
12	64.6	5.5
13	142.3	12.1
14	240.3	20.4
15	98.0	8.3
16	9.4	1.9

Table 4-72 Adhesive shear bond strength of GC ChemFil following one week of storage in distilled water.

Specimen number	Applied load (N)	Bond shear strength
1	134.2	11.4
2	132.9	11.3
3	157.1	13.3
4	299.3	25.4
5	120.8	10.3
6	034.9	3.0
7	268.5	22.8
8	36.3	3.1
9	67.1	5.7
10	80.1	6.8
11	114.1	9.7
12	84.6	7.2
13	75.2	6.4
14	88.6	7.5

Table 4-73 Adhesive shear bond strength of GC ChemFil + RGD following one week of storage in distilled water.

Specimen number	Applied load (N)	Bond shear strength
1	151.7	12.9
2	228.2	19.4
3	197.3	16.8
4	21.5	1.8
5	131.6	11.2
6	169.1	14.4
7	126.2	10.7
8	10.2	0.9
9	167.8	14.3
10	95.3	8.1
11	84.6	7.2
12	69.8	5.9
13	57.7	4.9
14	51.0	4.3
15	72.5	6.2

Table 4-74 Adhesive shear bond strength of GC ChemFil + Collagen I following one week of storage in distilled water.

Specimen number	Applied load (N)	Bond shear strength
1	38.9	3.3
2	92.6	7.9
3	60.4	5.1
4	205.4	17.4
5	85.9	7.3
6	59.1	5.0
7	123.5	10.5
8	63.1	5.4
9	115.4	9.8
10	24.2	2.1
11	120.8	10.3
12	32.2	2.7
13	0	0
14	68.5	5.8
15	38.9	3.3

Table 4-75 and figure 4-46 summary the mean and standard deviations of the adhesive Shear Bond Strengths.

Table 4-75 Adhesive shear Bond Strengths

Properties/Material	Shear bond Strength
GC Fuji VIII$_{GP}$	5.8 (6.7) n = 15
ChemFil Superior	10.3 (6.2) n = 14
ChemFil Superior + RGD	9.3 (5.5) n = 15
ChemFil Superior + Collagen I	6.3 (4.4) n = 15

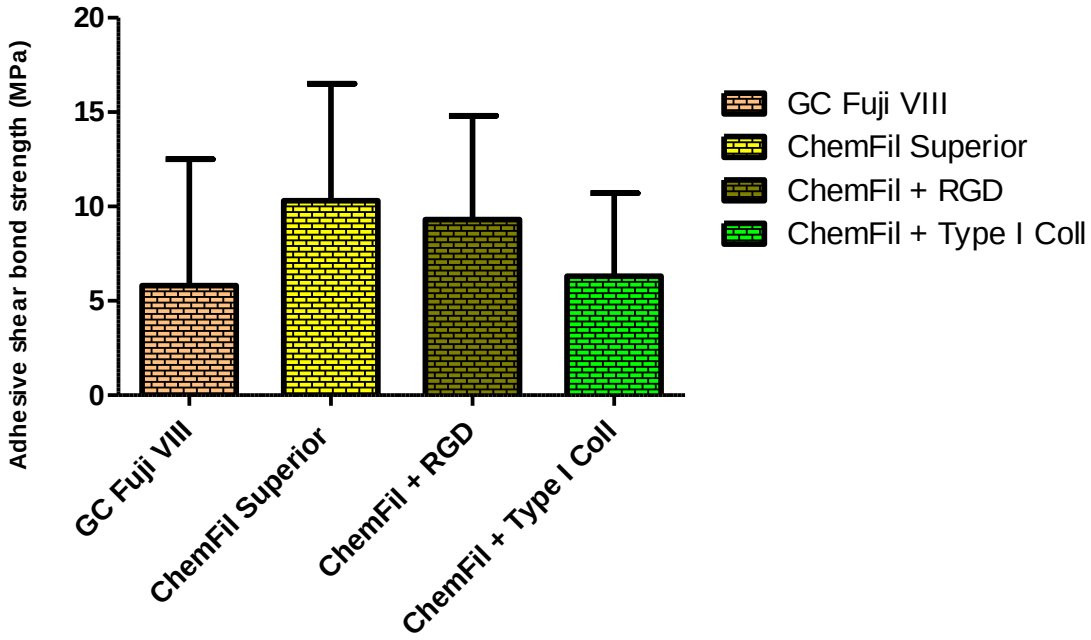

Figure 4-46 Adhesive shear Bond Strengths.

An analysis of variance revealed no statistically significant difference ($P > 0.05$) between values. It is clear therefore that the addition of the bioactive chemicals did not significantly impair this property.

5 DISCUSSION

5.1 QUESTIONNAIRE

In discussing the results of the questionnaire it is important to be clear why this was undertaken. Its main purpose was to see if anything new could be ascertained from practicing dentists that would advance treatments of root caries.

In broad terms no new radical treatment were found. Valuable ideas come from speaking to clinicians and in the field of caries treatment it should be borne in mind that the Hall technique (Innes et al., 2006), essentially sealing in caries with stainless steel crowns upon deciduous teeth to promote retention until exfoliation, was discovered by examining the practises of dentists. A secondary function of the questionnaire was to compare and contrast the root caries management practices of those in the UK compared to those in Libya where the author comes from.

It was pleasing to see the high return rate in Libya (60.00 %), but on the other hand a relatively poor return rate was obtained in the UK (34.35 %). This could have arisen because the questionnaires in Libya were personally issued and followed up by the author whereas in the UK the need to preserve anonymity did not allow such an approach nor individual follow up to boost return rates. Low response rates may be due to a lack of time to complete the questionnaire, no interest in the subject matter or concerns about the confidentiality of the responses (VanGeest et al., 2007). This last reason does not apply here for confidentiality of responses was stressed at the time of invitation. Robertson et al (2005) states that follow up letters can enhance return rate but had this been

done, on an individual basis, full ethical approval would have been required for the origin of the responses would have been known and it was felt that this increase in paperwork would have been off putting driving the response down. Notwithstanding this the study findings are interesting. It should be noted that the number of questionnaires that were undelivered in the UK (Table 4-1) agrees with the level reported in other dental surveys (Burke et al., 1994).

An interesting finding was that the Libyan respondents had been practising for a significantly shorter period of time than those from the UK. This can be explained by appreciating that the first dental school to open in Libya (Faculty of Dentistry, University of Benghazi) opened in 1974 and although there were some dentists who trained outside the country dentists in Libya in total were few in number (Metz, 1987).

As regards the age stated as being the most susceptible age to develop root caries (Table 4-2) both the UK and Libyan observations, that this condition is most likely to occur in the elderly, fits with established literature (Hassan and Omar, 2000, Steele and Sullivan, 2011).

It is interesting to note that in the UK the respondents indicated no difference between the sexes in relation to susceptibility to root caries. This was not the case in Libya [Table 4.3] however, with a significantly higher proportion of males said to be more susceptible. This could be because in that country it is perceived that females are more likely to attend the dentists. The lack, however of robust data from national statistics makes this statement purely opinion for the statistics held

by the health ministry of Libya are incomplete. Similar cultures e.g. Jordan also report this trend (Al Omari and Hamasha, 2005).

The finding that Libyan dentists use radiographic detection more frequently than their UK counterparts (Table 4-5) for root caries detection is perhaps a reflection of their training and also of the site of the caries. Libyan dentists (Table 4.6) report a high proportion of root caries affecting the interproximal surface compared to those in the UK. This observation agrees well with Hassan and Omar (2000) who conducted a survey of Libyan patients in Bangazi. In the UK the respondents indicated that root caries was more likely to be upon the labial surface.

It was more common in Libya to provide a restoration for a root caries lesion than in the UK (Table 4.7). This could be due to the fact that in Libya such lesions are more likely to be interproximal and there is relatively little patient follow up (Table 4-12, 4-13), perhaps due to the casual nature of attendance. This is borne out by the very low level of dietary advice apparently offered to Libyan patients (Table 4-8).

As regards to restoration both the UK and Libyan dentists used glass ionomer. In Libya however the proportion using composite was greater. According to recent guidelines (Momoi et al., 2012) composite should only be used where moisture control is optimal. Glass ionomer is said in this paper to be a viable alternative though it is acknowledged that for both materials there is a lack of clinical studies to support their use where moisture is present. The results of this survey (Table 4.10) indicated that bleeding from gingival tissues is a problem in the majority of respondents' experiences. This therefore warrants further investigations.

It should be remembered that a greater proportion of restorations placed in Libya were resin composite (Table 4.9). This material has no intrinsic ability to bond to tooth and is reliant upon acid etch and dentine bonding for its retention. Such mechanisms have no capacity to reform when broken. This contrasts to glass ionomer which can form and reform its bonds (Noble, 2012) to tooth substance and this property may explain, in part, the shorter life span of root caries restorations reported by the Libyan respondents. In addition, the experience of the Libyan operators was less than the UK and so this to may have been a factor.

It is interesting to note that finishing in Libya (Table 4.11) was deferred until the recall visit. This is unexpected for the preferred material in that country, although glass ionomer and not amenable to finishing at placement. represents a much lower proportion of the total number of restorations, and so as resin composite was used relatively more than in the UK a higher proportion of finishes at placement would have been expected This therefore warrants further work.

It is interesting to note that in the present study there is no significant difference in the responses from the two countries in terms of relative importance of life style events associated with root caries development (Table 4.15). It is however surprising to note that diet is considered to be a relatively unimportant factor for this provides the substrate required to develop caries.

In relation to factors considered by the respondents to be risks important for the development of root caries there are significant differences between the responses of the countries with regard to Degree of crowding, Cigarette smoking, Total amount of sugars consumed, Frequency of sugar intake, Active periodontal disease, Consumption of alcohol, Poor crown margins, Gingival recession, Reduce salivary flow, Presence of erosion and presence of abrasion cavity (Table 4.16). In order to

compare these to the other studies in the literature (McCombes, 1999) it is necessary to devise a system of ranking. One such method, adopted here, is to multiply the percentage by the rank of importance attached to each factor, for response levels of 1= very important, 2= quite important and 3= fairly important, giving weighting of 3 to a rank of 1, 2 to a rank of 2, and 1 to a rank of 3. When this empirical comparison is carried out for the findings of the present study and also to that of the work of McCombes (1999) in an endeavour to standardise the comparison (Table 5.1 results).

It should be noted that in the preparation of this table the ranks of McCombes (1999)are decreased by one for in the present study the effect of altered bone contour after periodontal therapy was not examined.

A crude assessment of the differences in responses is gained by subtracting the resultant weighted rankings of the studies being compared, to give a numerical value. Where this is zero there is 100 % agreement between the studies. Empirically a differences of 4 or greater is worthy of comment. On this basis it is interesting to note the general broad agreement between the present work and that of MaCombes (1999) with the exceptions of poor general health, cigarette smoking, poor crown margins and overhanging restoration. In case of poor general health and cigarette smoking there were considered to be lesser importance by McCombes whereas the opposite was true for poor crown margin and overhanging restorations compared to the present study. An explanation for this is not immediately obvious for the questionnaire contents were similar. Such an observation may be due to possible changes in the perceptions of dentists about root caries lesion since 1999 the year in which the McCombes study was conducted.

Table 5-1 Comparison of relative importance of risk factors for root caries from the present UK study and that of McCombes (1999).

Risk factors	Present UK study	McCombes	Level of agreement
Oral hygiene state	1	3	2
Frequency of sugar intake	2	2	0
Reduce salivary flow	3	1	2
Total amount of sugars consumed	4	5	1
Mental disability/senility	5	4	1
Physical disability	6	8	2
Poor general health	7	13	6
Cigarette smoking	8	17	9
Presence of a partial denture	9	9	0
Consumption of fizzy drink	10	10	0
Active periodontal disease	11	11	0
Gingival recession	12	12	0
Poor crown margins	13	7	9
Overhanging restoration	14	6	8
Degree of crowding	15	15	0
Presence of erosion	16	14	2
Consumption of alcohol	17	18	1
Number of teeth present	18	16	2
Presence of abrasion cavity	19	19	0

Body of table gives relative ranking of factors importance. Level of agreement between studies being compared is 100% if value column is 0 and is 0% if difference is 19.

Table 5-2 compares the relative ranking afforded to the risk factors by the respondents, both Libyan and UK, in the present study. It is clear that there are many differences with dentists in Libya ranking the importance of active periodontal disease, gingival recession, crowding and alcohol consumption higher than the UK respondents. In contrast the UK respondents awarded higher significance to dietary, physical disability, partial denture wearing, fizzy drink consumption and detective crown margins. From the observations of the author this perhaps reflects the greater emphasis upon prevention in the UK and the greater levels of untreated dental disease in Libya. The importance of alcohol consumption in Libya could be due to religions and culture beliefs prohibiting its consumption.

Table 5-2 Comparison of relative importance of risk factors for root caries from the UK and Libya study

Risk factors	UK	Libya	Level of agreement
Oral hygiene state	1	1	**0**
Frequency of sugar intake	2	9	**7**
Reduce salivary flow	3	4	**1**
Total amount of sugars consumed	4	15	**11**
Mental disability/senility	5	5	**0**
Physical disability	6	12	**6**
Poor general health	7	11	**5**
Cigarette smoking	8	10	**2**
Presence of a partial denture	9	17	**12**
Consumption of fizzy drink	10	18	**8**
Active periodontal disease	11	2	**7**
Gingival recession	12	3	**9**
Poor crown margins	13	7	**6**
Overhanging restoration	14	13	**1**
Degree of crowding	15	6	**9**
Presence of erosion	16	16	**0**
Consumption of alcohol	17	8	**9**
Number of teeth present	18	19	**1**
Presence of abrasion cavity	19	14	**5**

body of table gives relative ranking of factors importance. Level of agreement between studies being compared is 100% if value column is 0 and is 0% if difference is 19.

5.1.1 CONCLUSION

In conclusion attitudes and beliefs about root caries between and Libyan respondents exhibit some commonality about differ in the emphasis on prevention. Similar treatments are carried out when prevention fails in both countries the decision to do so however would appear to be based upon a different weighting of risk factors.

5.2 BASELINE PROPERTIES OF THE GLASS IONOMERS USED IN THE STUDY

This laboratory work sought to investigate the potential to modify existing glass ionomers to promote cellular adhesion to improve the treatment of root caries. In order to establish if modification had an adverse effect upon material properties baseline property values were determined of the unmodified materials using recognised laboratory testing techniques.

It should be noted in preparing the specimens for testing a clinically realistic mixing regime was adopted. This was because it was it envisaged that the material, and any additions to it, would be placed immediately into the root surface cavity, and thus contrary to the manufacturer's instructions would be exposed to blood and moisture. It was to part simulate this that no water impervious coatings were applied to the specimens prior to storage.

5.2.1 MATERIAL SELECTION

Two different glass ionomers were selected to be modified. These were both commercially available and presented in powder and liquid format for hand mixing. These were favoured over encapsulated materials for they more readily permitted modification of their constituents by the inclusion of biological additives. One of these was a conventional glass ionomer (ChemFil Superior), in freeze dried form, activated by the addition of water to bring about acid ionisation. This product has been on the market since 2005. The other material was Fuji VIII and this is recommended by the manufacturer for treatment of class V cavities including root caries lesion (GC, 2013). It is a more recently developed material

and has a resin component (HEMA) to improve mechanical properties. It is chemically set and like ChemFil Superior involves no light curing.

5.2.2 TESTING METHODS AND BASELINE RESULTS

The properties evaluated were those thought to be most relevant to clinical success. Although not reported here many attempts were made to obtain a value of flexural fatigue strength for both materials. Due however to the often premature failures of ChemFil superior specimens, not even making one flexural fatigue cycle, compared to Fuji VIII specimens flexural fatigue was not tested. A search of the literature failed to identify any studies that report flexural fatigue properties of ChemFil superior perhaps reinforcing the difficulties experienced.

In order to simplify the comparison of the baseline property values determined in the present study to those reported by others in the literature Table 5-3 a,b and Table 5-4 summarises the values obtained by others. It should be noted however that the materials and storage regimes differ so a direct comparison cannot be made. The values however serve as good source of ball park figures to compare the present work against.

Table 5-3 (a, b) The properties of glass ionomers from other laboratory studies that examined some the same physical properties as the present study.

Authors	Materials	Flexural strength (MPa)	Compressive strength (MPa)	Microhardness	Flexural fatigue limit (MPa)	storage time
(Prosser et al., 1986)	**Experimental**	16.4	-	-	-	1 d
	Experimental	7.6	-	-	-	1d
(Ewoldsen, 1997)	Fuji IX	-	193±13	-	-	
(Xie et al., 2000)	**Ketac Fil**	22.6 ±2.5(3-PB)	251.2±10 .0	176.8±12.8 (KH)	-	7 d
	Ketac Molar	21.2±3.1 (3-PB)	301.3± 10.1	108.8±12.8(KH)	-	7 d
	Fuji II	26.1± 3.9(3-PB)	202.0± 10.0	83.15± 8.20(KH)	-	7 d
(Iazzetti et al., 2001)	**Fuji IX**	22.6/15.4 (3-PB)		-	-	1 d/7 d
(Lucksanasombool et al., 2002)	**Fuji IX**	29.2 (BB)	211	-	-	1 h
(Bapna et al., 2002)	**Fuji II**	30.8±7.5 /23.0 ±5.1(3-PB)	-	-	-	1 d/9 m
	Fuji II	47.1±5.4/21.4±9.8 (3-PB)	-	-	-	1 d/9 m
	Fuji II	17.8±8/14.6±8.7 (3-PB)	-	-	-	1d/9 m
(Fleming and Zala, 2003)	**Fuji IX GP**	-	87.9/67.9	-	-	1d
	Ketac Fil Plus	-	72.7/62.0	-	-	1 d
	ChemFlex	-	84.3/68.9	-	-	1 d
(Lohbauer et al., 2003)		19.7/33.0/35.2/3.7 (4-PB)	-	-	13.2/17.5/21.4/29.4/-	24 h/8 d/30 d/90 d
(Sunnegårdh-Grönberg et al., 2003)	**Chemfil Superior**	32±8/40±10/46±4				1d/1w/2w
(McKenzie et al., 2003)	**Chemfil Superior**	52.8±14.1/47.2±9.1/54.7±10.7/49.4±3 .1/43.1±2.1/50.73.2± (BB)	177.1±14.7/188.9±23.1/182.7±22.9/ 148.1±16.3 /192.0±24.5 /184.1±25.9	-		1 d/1 w/1 m/3 m/6 m/1 y
	Aqua Cem	29.8±6.9/44.1±2.6/31.8 ±4.4/31.9± 3.8/45.0±3.8/40.7±3.8 (BB)	130.5±22.3/132.2±16.2/154.7±18/150.6 ± 25.4/214.8±18/192.1±39	-		1 d/1 w/1 m/3 m/6 m/1 y
	ChemFlex	67.2±5.1/51.2±11.5/49.0±13.2/58.9±7 .8/54.2±5.3/66.1±10 (BB)	197.8±12.5/147.2±43.5/227.1 ±18.6/232.5±22.5/268.2±24.9/240.1± 39.2	-		1 d/1 w/1 m/3 m/6 m/1 y

1FS: flexural strength; CS: compressive strength; 3-PB: three-point-bending; 4-PB: four-point-bending; BB: biaxial bending; KH: knoop hardness; VH: Vickers hardness; ws: wet storage; as: air storage; wc: wet cyclic.

Table 5-3 b.

Authors	Materials	Flexural strength (MPa)	Compressive strength (MPa)	Microhardness	Flexural fatigue limit (MPa)	storage time
(Pamir et al., 2005)	**Chemfil Superior**	-	148.30±9.63	49.89± (VH)		28 d
(Peez and Frank, 2006)	**Ketac Molar**	51 ±5 (3-PB)	244±9	-		1d
	Fuji IX	42± 4(3-PB)	236±28	-		1d
	Vitro Molar	48±3 (3-PB)	141±15	-		1d
	Vidrion R	38 ± 2(3-PB)	175±11	-		1d
	Ionofil Molar	36 ±9 (3-PB)	196±12	-		1d
(Irie et al., 2008)	**Fuji IX GP**	1.8/29.2 (3-PB)	-	-		immediate/1d
	FX-II	1.7/17.3 (3-PB)	-	-		immediate/1d
	Ketac Molar	1.9/19.3 (3-PB)	-	-		immediate/1d
	Fuji II	2.0/15.3 (3-PB)	-	-		immediate/1d
(Moshaverinia et al., 2008a)	**Fuji II**	14.8 (BB)	161	-		1h
(Türkün et al., 2008)	**ChemFil Superior**	43,06±9.92 (BB)	211.1±14.1	**57.23(VH)**		1d
(Dowling and Fleming, 2009)	**Ketac Fil Plus**	-	129±19	-		1d
	Fuji II	-	131±14	-		1d
	ChemFil Superior	-	132±18	-		1d

1FS: flexural strength; CS: compressive strength; 3-PB: three-point-bending; 4-PB: four-point-bending; BB: biaxial bending; KH: knoop hardness; VH: Vickers hardness; ws: wet storage; as: air storage; wc: wet cyclic.

Table 5-4 The properties of glass ionomers from other laboratory studies that examined the same physical properties as the present study.

Authors	Materials	Enamel Bond	Dentin Bond	Diametral compressive	Diametral fatigue	storage time
(Ewoldsen, 1997)	**Fuji IX**	2.6 ± 0.8	3.8 ±1.1			
(Sunnegårdh-Grönberg et al., 2003)	**ChemFil Superior**					
(Türkün et al., 2008)	**ChemFil Superior**			12.89±3.24		1d

5.2.2.1 *SHORE HARDNESS*

In this work Shore hardness was determined. This test was selected over the more usually performed Vickers hardens test for it did not require the specimen surface to be highly polished in order to visualise the diamond indent. At the time of planning the study it was not known how the incorporation of additives would affect polishability of the samples and it seemed prudent therefore to adopt a relative ease of penetration test, such as afforded by the Shore tester, to enable a hardness assessment to be made. A downside of this approach is that the results presented here cannot be compared to other literature values for no other workers have employed this test for glass ionomers.

5.2.2.2 *COMPRESSIVE STRENGTHS-LONGITUDINAL AND DIAMETRAL*

Both these properties were determined using an Instron universal testing machine at 1mm min^{-1}. As observed by others the ends of longitudinal compressive specimens must be lapped flat otherwise edge effects lead to premature failure of the specimen by facilitating crack initiation and propagation (Cho and Garant, 2000) . It is however not possible to precisely lap flat and so in the present investigation this was not carried out. To therefore obtain a more meaningful assessment a diametral compressive strength test was also performed. This test avoids edge effects and is the test of choice for brittle materials as used in this study (Darvell, 2009). The baseline results obtained in this study are in general agreement with other values published in the literature. Thought it is acknowledged they are lower this is in all likelihood due to both the mixing and storage regime as stated in the introductory paragraph-5.2 (Baseline properties of

the glass ionomers used in the study). Both of these properties are considered to be of relevance to clinical function (Cho and Garant, 1989). Mastication applies compressive forces as tested here by the compressive strength whereas the diametral compressive test replicates in some way clinical failures due to tensile stress (Freeman and ten Cate, 1971). In this thesis convention has been broken for the term compressive diametral has been used to indicate the experimental set up and direction of applied force. Conventionally such a test is called a diametral tensile test for the compression plates, either side of the specimen; apply largely tensile forces with also an element of compression. The latter has been identified as a factor that to a degree prevents the propagation of the tensile crack (Darvell, 2009). True tensile testing of brittle materials, such as glass ionomer, is not practicable and was internationally agreed some time ago to therefore adopt the diametral tensile strength test as a means of assessing this property (British Standards Institution, 1981).

5.2.2.3 *3 POINT FLEXURAL STRENGTH*

The jig used to determine the three point flexural strength of the materials tested contained within it two cylindrical rollers upon which the specimens sat. This was preferable to point contacts for these linked setting stress concentration which would enhance crack propagation.

Karbhari and Wang (2007) have commented that the ratio of support separation to the specimen depth should be 10 in order to ensure that bending moments dominate over shear forces this practice, and the experimental set up, conforms to ISO 9917 (International Organization for Standardization, 1998) . This was

achieved by this experimental set up. The baseline values obtained (table 4-57) are similar to other comparable materials in the literature (table 5.3 a, b) although the author would have expected the value for Fuji VIII to be higher due to its resin reinforcement.

5.2.2.4 *DIAMETRAL COMPRESSIVE FATIGUE*

Although this work has examined many static properties it should not be forgotten that in its lifetime a dental restoration will be subjected to many cyclical forces. Theses cumulatively can cause a material to fail. It is therefore potentially misleading to report static properties alone.

For this reason the diametral compressive fatigue limit of the materials alone and with additives was determined.

In order to minimise edge effects this property was favoured over compressive fatigue and for practical reasons (see section 5.2.2.2 COMPRESSIVE STRENGTHS-LONGITUDINAL AND DIAMETRAL) the flexural fatigue was not determined. The method used to determine this property is widely accepted and seeks to relate the number of cycles to failure versus the applied stress (Draughn, 1979). The definition of the number of cycles to be survived was set empirically at 1500 cycles so that work was achievable in a realistic laboratory time frame. As all materials tested followed the same testing regime this permitted ready comparison. Surprisingly a search of the literature revealed no diametral fatigue limits against which the present values can be compared. Perhaps unsurprisingly the fatigue limit obtained for unmodified Fuji VIII was statistically significantly higher than for the conventional glass ionomer ChemFil superior (Tables 4-63, 4.64). This can be attributed to the resin present in the former material. When the fatigue limits were compared to the

mean diametral compressive strength values they are lower as would be expected. The biological additions both significantly increased the fatigue strength of ChemFil superior.

5.2.2.5 *ADHESIVE SHEAR BOND STRENTGH TO BOVINE ENAMEL*

In this work an assessment of the bond of the glass ionomer to tooth substance was made. Self adhesion is a unique property of the glass ionomers studied here. It is considered important to the proposed application of the modified materials of this work for it will provide a marginal seal and thus, if durable prevent the ingress of bacteria and of recurrent caries. Its determination is however, controversial in respect of testing method and tooth substrate used.

This study utilised macro testing (one tooth per test) compared to micro testing (sections of the same tooth used in multiple tests). This was because we lacked the facilities to carry out micro testing. In a critical review of bond strength test methods Armstrong et al., (2010) supported the continuation of macro testing. In terms of geometry and technique of testing it is acknowledged, as reviewed by Van Noort et al., (1989) that the results of such testing are affected by the specimen geometry, loading configuration and material stiffness. In the present work the first two of these were controlled by standardization of the methods but it is acknowledged that the material stiffness could have been affected by the additions.

Due to the relatively easier collection of bovine teeth as compared to human teeth, the former tissue was used for testing. Some have indicated that the bond strengths to bovine enamel are in the range of 21% to 44% less than to human enamel (Oesterle et al., 1998).

An examination of the baseline bond strengths of the materials in unmodified form (table 4-57) the values obtained, accepting that they are not directly comparable by virtue of experimental set up and tissues used, mirror those reported by others (Table 5-4).

5.2.2.6 *BIAXIAL FLXURAL STRENGTH*

The materials investigated in this study are brittle. By their nature they contain flaws and such inclusions are exacerbated by handling and mixing. The impact of these upon the physical property of biaxial flexural strength is a useful test for it is said to be more searching for defects than a uni-axial test (Börger et al., 2002). The testing geometry used in this study, comprising a circular disc supported upon three balls, has been shown to be

- Insensitive for a specimen whose surface is rough.
- Insensitive to edge effects that could act as stress concentration precipitating early failure (Börger et al., 2002).

The calculation uses the Poisson's Ratio. This is defined as the Ratio of lateral strain to axial strain in an axial loaded specimen. it has been determined previously by Akinmade and Nicholson (1993b) as 0.35 for glass ionomer, the value used here, though in a subsequent paper they quote a value of 0.27 (Akinmade and Nicholson, 1995). This work treats all results the same way and so this difference in terms of relative performance is of no relevance.

The baseline values obtained for biaxial flexural strength in this work are in good agreement to those reported by others (McKenzie et al., 2003)

5.3 BIOACTIVE ADDITIONS TO GLASS POLYALKENOATE CEMENTS

Materials used for cell assays were washed in HBSS for 24 hours change pH from 2.2 to 6.6 at low pH the cells would die (Smith and Ruse, 1986). The washed materials were analysed using a number of techniques to analyse cell viability including microscope observation and MTT assay. In order to investigate a number of materials quickly and easily we used a simple observational technique similar to work by (Caughman et al., 1990). These involved observing cells close and away from the materials. Cells growing on the materials could not be seen. After initial experiments work focused on MMT assay to check the ability of cells to attach and grow on the materials. Immunocytochemistry and Western blotting to investigate the express of Vimentin. Vimentin is intermediate protein has a molecular weight of 57 kD and is used as primarily a fibroblast marker (Ivaska et al., 2007). It should be pointed out in light of the laboratory study of the effects of the materials in unmodified state no bioactive addition were made to GC Fuji VIII. This material inhibited cell growth. It contains hydroxyl ethyl methacrylate (HEMA) and others have observed that it alters the morphology of human gingival fibroblasts and interferes with the presence of Type I collagen protein (Falconi et al., 2007). It therefore was inappropriate to make additions to this material in order to explore the potential of this material to promote cellular adhesion. This study indicates that the MM1 cells appear to become more fibroblastic in response to GI will added Type I collagen. When applied close to the material Type I collagen and RGD produced a better cell viability than Emdogain as addressed by both microscopy and MTT assay.

5.4 THE PHYSICAL PROPERTIES OF THE GLASS POLYALKENOATE CEMENTS FOLLOWING BIOACTIVE ADDITIONS

With the exception of shear bond strength and 3 points flexural strength all the properties tested improved significantly, compared to the baseline values, for the samples of ChemFil Superior to which 0.1% collagen Type I was added. As regards additions of RGD similar improvements were observed for all properties except shear bond strength and hardness.

In relation to shear bond strength, although no improvements in bond strength were observed, the addition did not statistically impair this property. Perhaps this is not surprising as the additions do not have an obvious mechanism for degrading or improving this bond.

Glass ionomer adheres to tooth substance by the formation of chemical linkages with the calcium of the tooth substance. They cannot roughen the surface of the tooth in order to promote micromechanical attachment as they are not of sufficiently low pH.

It has been observed by others that the pH of a setting conventional glass ionomer (ChemFil) changes with time commencing around 2.2 and increasing to 6.2 after 1440 minutes (Smith and Ruse, 1986). This range of pH value is conducive for the collagen Type I addition to form molecular aggregates, fibrils and ultimately fibres (Harris et al., 2013). Previously the formation of 70 ~nm collagen granules upon glass ionomers has been observed in SEM and AFM studies (Chang et al., 2009). These have the potential to facilitate cellular attachment to the glass ionomer and also the fibrils have the potential to bring about fibre reinforcement of the glass

ionomer providing they make attachment to the material. Evidence for this is provided by the improvement in physical properties, where Type I collagen was added, but further work is required to test this hypothesis.

In relation to the improvements in properties seen when RGD was added to the conventional glass ionomer an exact mechanism has not been found in this work. It is however possible that the aminoacids (Arginine,glycine, and aspartic acid) in some way become involved in the setting reaction. They have the capacity to crosslink by ionic bridges to the Calcium and Aluminium of the glass particles and to bond with the materials particles and also by dipole dipole interaction and/or hydrogen bonding with materials parent acid. Further work would be required to determine if this was the case.

molecular structure indicates this is possible. Others have demonstrated that RGD can promote adhesion and osteoblast activates in relation to a pure calcium phosphate cement (Wu et al., 2013). It is worth noting that in this application the biological scaffold Chitosan improved the calcium phosphates properties by a fibre reinforcing mechanism (Wu et al., 2013). It therefore does not seem as remote a possibility as the first though suggests.

6 PRINCIPLE FINDING AND FURTHER WORK

The principle findings of this study are;

- There was no different between Libya and the UK in terms the teeth most commonly affected by root caries.
- Root caries in childhood was not reported by either country but a greater proportion of Libyan respondents reported root caries in adulthood (26.49% Libya of 2.25% UK).
- A greater proportion of patients were considered by the respondents to be at risk of root surface caries in the UK.
- In both countries the most common method of detecting root surface caries was visual. In Libya however radiographic detection was used more commonly than in the UK (34.86% Libya cf 22.03 UK).
- In both countries root surface caries was most common on the buccal surface but in Libya proportionally more reports of root caries affecting the interproximal surface were made.
- Restoration of the caries lesions rather than preventive methods was undertaken more in Libya
- The diet of patients in the UK influenced to a greater extent the management of the root caries (UK= 23.86% versus Libya 08.11%)
- Gingival bleeding was a common problem encountered in both countries on restoring root caries lesions

- In the UK it was most common to follow up root caries restorations (UK 66.67% cf. Libya 29.52%) every six months (UK 80%).
- Proportionately more root caries restoration failed in the first year in Libya (15.38% Libya cf. 0% UK). Longer survival times were reported in the UK.
- In both countries giving up smoking was the factor most considered to give rise to root surface caries. Surprisingly diet was considered by the respondents to be relatively unimportant (UK 4.44%, Libya 0%)

- When applied close to the material collagen and RGD produced a better cell viability than Emdogain as addressed by both microscopy and MTT assay.
- The addition of Type I collagen to ChemFil Superior increases the expression of Vimentin as determined by Immunocytochemistry and western blotting indicating that the cells have become more fibroblastic. This increase was not seen to the same level with emdogain.

The Further work;

- To determine if the successful bioactive additions may improve abrasion resistance
- To determine if the bioactive addition if the bioactive additions maintain with time their beneficial effects.
- to chemically bond the bioactive materials to the ChemFil superior

- Use electron scan microscopic techniques to determine the mechanism of Type I collagen and RGD interaction with glass ionomer.
- Transfer the finding to clinical application.

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8 APPENDICES

Appendix A- The ethical approval

NHS Fife NHS Forth Valley NHS Tayside

East of Scotland Tayside Research Ethics Service

Research Ethics Service Office
Residency Block
Level 2
Ninewells Hospital & Medical School
DUNDEE
DD1 9SY

Date: June 10th 2010
Your Ref:
Our Ref:
Enquiries to: Mrs Caroline Ackland
Extension: Ninewells extension 32588
Direct Line: 01382 632588
Email: caroline.ackland@nhs.net

Dear Dr Chadwick

Re: Proposed Postal Questionnaire on Root Surface Caries

You have sought advice from the Research Ethics Office on the above project. The Research Ethics Co-ordinators and I have considered this and can advise that this does not require ethical review under the terms of the Governance Arrangement for Research Ethics Committees (GAfREC) in the UK. The advice is based on the following documentation provided to us:

Document	Version	Date
Letter of Invitation	Not specified	Not specified
Root Surface Caries Questionnaire	Not specified	Not specified
Covering letter	Not specified	31/05/2010

- You are undertaking an anonymous questionnaire survey

Please note that this advice is issued on behalf of the Research Ethics Service Office and does not constitute an opinion of a Research Ethics Committee (REC). It is intended to satisfy journal editors and conference organisers, who may require evidence of consideration of the need for ethical review prior to publication or presentation of your results.

You should keep a copy of this letter within your project file.

Yours sincerely,

Caroline

Caroline Ackland (Mrs)
Scientific Officer to the East of Scotland Research Ethics Service

Appendix B -Root Surface Caries Questionnaire

Root Surface Caries Questionnaire

1. How long have you been practising dentistry?

2. From your clinical experience please indicate at what age people are most susceptible to root surface caries?

    - Childhood
    - Adulthood
    - Elderly

3- In your experience please indicate who are more susceptible to root caries?

    - Males
    - Females
    - No difference between males and females

4- In your experience which age group is most susceptible to root surface caries?

    - 20- 30 year
    - 31- 40 year
    - 41- 50 year
    - 51- 60 year
    - > 60 years

5- Does your practice base have a large proportion of patients prone to root surface caries?

    - Yes
    - No

6- How do you usually detect root surface caries?

- Visually (inspection)
- Tactile (probing)
- Dental radiographs (X-ray)
- Others (please specify in the box)

7- In your experience which surface of a tooth is most commonly infected with root caries?

- Labial (Buccal) surface
- Interproximal surface
- Palatal (Lingual) surface

8- In your experience which anterior tooth/teeth is most commonly affected by root surface caries? Circle those that apply

3	2	1	1	2	3
3	2	1	1	2	3

9- In your experience which posterior tooth/teeth is most commonly affected by root surface caries? Circle those that apply

8	7	6	5	4	4	5	6	7	8
8	7	6	5	4	4	5	6	7	8

10- Which method(s) do you usually use to manage root surface caries?

- Monitoring with prevention instruction
- Dietary advice
- Topical Fluoride
- Restoration
- Ozone
- Others (please specify in the box)

11- In deciding which method you use to manage root surface caries tick those factors that influence your selection of the management method?

- Patients oral hygiene
- Diet
- Patients age
- Tooth type
- Severity of the lesion
- Other (please specify in the box)

12- Which restorative material do you most commonly use to restore a tooth with root surface caries?

- Amalgam
- Glass ionomer
- Composite
- Compomer
- Others (please specify in the box)

13- What other restorative materials do you sometimes use to restore a tooth with root surface caries?

- Amalgam
- Glass ionomer
- Composite
- Compomer
- Others (please specify in the box)

14- Is bleeding from the gingival tissues normally a problem in restoring root surface caries?

- Never
- Sometimes
- Frequently

15- After you restore a tooth with root caries, what method of finishing do you use for the restoration?

- Hand finishing (sharp knives or scalers) at placement visit
- Rotary finishing at placement visit
- Hand finishing (sharp knives or scalers) at recall visit
- Rotary finishing at recall visit
- Others (please specify in the box)

16- After you treat the root caries do you follow up your patients?

- Yes. if yes specify for how long in the box below

- No
- Sometimes

Follow up duration =

17- In your experience what is the average lifespan of the restoration you most commonly use for the restoration of root caries?

- Less than a year
- 1 to 5 years
- More than 5 years

18- In your experience which lifestyle events are associated with root surface caries development? (tick all that apply)

- Bereavement
- Retirement
- Giving up smoking
- Loss of job
- Others (please specify in the box)

19- From your experience, indicate which of the following factors you feel are important in the development of root caries. Please circle one number on each line, where 1 = very important, 2 = quite important, 3 = fairly important, 4 = not at all important.

- Number of teeth present 1 2 3 4

• Degree of crowding	1	2	3	4
• Presence of a partial denture	1	2	3	4
• Cigarette smoking	1	2	3	4
• Total amount of sugars consumed	1	2	3	4
• Frequency of sugar intake	1	2	3	4
• Oral hygiene state	1	2	3	4
• Physical disability	1	2	3	4
• Mental disability/senility	1	2	3	4
• Active periodontal disease	1	2	3	4
• Consumption of alcohol	1	2	3	4
• Consumption of fizzy drinks	1	2	3	4
• Overhanging restoration	1	2	3	4
• Poor crown margins	1	2	3	4
• Gingival recession	1	2	3	4
• Reduce salivary flow	1	2	3	4
• Presence of erosion	1	2	3	4
• Presence of abrasion cavity	1	2	3	4
• Poor general health	1	2	3	4

Appendix C The cover letter

UNIVERSITY OF DUNDEE

Dental School

UNIT OF RESTORATIVE DENTAL CARE AND CLINICAL DENTAL SCIENCES
Sections of Operative Dentistry, Fixed Prosthodontics and Endodontology
Section of Integrated Oral Care

1st July 2010

RE: Root Surface Caries

Dear Colleague

I am a dentist undertaking a research project, under the supervision of Dr R Graham Chadwick that seeks to develop novel approaches to the management of root surface caries. This is for the degree of Doctor of Philosophy.

I should be grateful if you would complete the enclosed questionnaire and return it in the pre-paid envelope. Your name was identified at random from The Dentists' Register and your return will be anonymous for no hidden codes are embedded in any part of the questionnaire paper work.

Thank you for your consideration.

Yours sincerely,

Abdurahman Salem

BSc in Dentistry and Oral Surgery Al-Fateh University, Libya, MSc in Bioengineering University of Strathclyde, Glasgow.

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Appendix D- The raw data obtained from ranking the images of oral mucosa fibroblast cells cultured around ChemFil superior and GC Fuji VIII for 21 days by ten observers.

Away 24 hrs	Control	ChemFil Superior	Fuji VIII
1	1	1	3
2	1	1	3
3	1	1	3
4	1	3	4
5	1	1	4
6	1	2	3
7	2	2	4
8	2	2	3
9	2	3	4
10	2	2	4

Close 24hrs	Control	ChemFil Superior	Fuji VIII
1	1	1	3
2	1	2	3
3	1	1	3
4	2	2	3
5	2	2	4
6	2	2	3
7	3	3	4
8	2	2	3
9	2	2	4
10	2	2	4

Away 3 days	Control	ChemFil Superior	Fuji VIII
1	1	1	2
2	1	2	2
3	1	1	2
4	1	1	2
5	1	1	2
6	1	1	2
7	2	1	2
8	2	2	2
9	2	2	3
10	2	2	3

Close 3 days	Control	ChemFil Superior	Fuji VIII
1	1	1	4
2	1	2	3
3	1	1	3
4	1	2	4
5	2	2	4
6	1	2	4
7	3	3	4
8	2	2	3
9	2	2	4
10	2	2	4

Away 6 days	Control	ChemFil Superior	Fuji VIII
1	1	1	2
2	1	2	3
3	2	2	2
4	1	1	2
5	1	2	3
6	1	1	2
7	1	2	2
8	1	2	2
9	1	2	3
10	1	1	2

Close 6 days	Control	ChemFil Superior	Fuji VIII
1	1	2	4
2	1	3	4
3	2	1	3
4	2	2	4
5	2	3	4
6	1	2	3
7	1	3	4
8	1	2	3
9	1	2	4
10	1	3	4

Away 9 days	Control	ChemFil Superior	Fuji VIII
1	1	1	3
2	1	2	4
3	2	2	3
4	1	1	3
5	1	1	4
6	2	1	3
7	1	3	4
8	1	2	3
9	1	2	4
10	1	1	4

Close 9 days	Control	ChemFil Superior	Fuji VIII
1	1	3	4
2	1	3	4
3	2	2	4
4	1	3	4
5	2	3	4
6	1	2	3
7	1	3	4
8	1	3	4
9	1	3	4
10	1	3	4

Away 12 days	Control	ChemFil Superior	Fuji VIII
1	1	2	3
2	1	2	4
3	2	2	4
4	1	2	4
5	1	3	4
6	1	2	3
7	1	3	4
8	1	3	3
9	1	3	4
10	1	3	4

Close 12 days	Control	ChemFil Superior	Fuji VIII
1	1	3	4
2	1	3	4
3	2	2	4
4	1	3	4
5	2	4	4
6	1	3	4
7	1	4	4
8	1	3	4
9	1	3	4
10	1	3	4

Away 15 days	Control	ChemFil Superior	Fuji VIII
1	1	2	3
2	1	3	4
3	2	2	4
4	1	2	3
5	1	3	4
6	1	2	2
7	1	3	4
8	1	2	3
9	1	3	4
10	1	3	4

Close 15 days	Control	ChemFil Superior	Fuji VIII
1	1	3	4
2	1	3	4
3	2	3	4
4	1	3	4
5	1	4	4
6	1	3	4
7	1	4	4
8	1	3	4
9	1	4	4
10	1	3	4

Away 18 days	Control	ChemFil Superior	Fuji VIII
1	1	2	4
2	1	3	4
3	2	2	4
4	1	2	4
5	1	3	4
6	1	2	3
7	1	3	4
8	1	2	3
9	1	3	4
10	1	3	4

Close 18 days	Control	ChemFil Superior	Fuji VIII
1	1	3	4
2	1	4	4
3	2	3	4
4	1	3	4
5	1	3	5
6	1	3	4
7	1	4	4
8	1	3	4
9	1	4	4
10	1	3	4

Away 21 days	Control	ChemFil Superior	Fuji VIII
1	1	3	4
2	1	3	4
3	2	3	4
4	1	3	4
5	1	3	4
6	1	3	4
7	1	3	4
8	1	3	3
9	1	4	4
10	1	3	4

Close 21 days	Control	ChemFil Superior	Fuji VIII
1	1	3	4
2	1	4	4
3	2	3	4
4	1	3	4
5	1	4	4
6	1	3	4
7	1	4	4
8	1	3	4
9	1	4	4
10	1	4	4

Appendix E- The raw data obtained from ranking the images of oral mucosa fibroblast close and away from unmodified and bio-modified ChemFil superior with different concentration of Type I collagen for 21 days by ten observers.

AWAY 24hr	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	1	1	1
2	2	2	2
3	1	1	2
4	3	3	2
5	2	2	2
6	1	1	1
7	3	2	2
8	2	2	2
9	2	2	2
10	2	2	2

Close 24hrs	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	1	2	1
2	2	3	2
3	2	3	2
4	3	3	3
5	2	2	2
6	2	2	3
7	3	3	3
8	2	2	2
9	2	3	3
10	2	3	3

AWAY 3 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	1	1	1
2	2	2	2
3	1	1	2
4	3	3	3
5	2	2	3
6	1	1	1
7	3	3	3
8	2	2	2
9	2	2	2
10	2	2	3

Close 3 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	2	3	1
2	2	3	2
3	2	3	2
4	3	3	2
5	2	3	3
6	2	3	1
7	3	4	3
8	2	3	2
9	2	3	2
10	2	3	2

Away 6 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	1	1	1
2	1	1	2
3	2	2	2
4	3	2	3
5	1	1	2
6	1	1	2
7	3	1	3
8	1	2	2
9	2	2	2
10	1	1	2

Close 6 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	2	3	1
2	2	3	2
3	2	3	2
4	3	3	2
5	2	3	2
6	1	2	2
7	2	3	2
8	2	3	2
9	2	3	2
10	2	3	2

Away 9 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	1	1	1
2	1	1	1
3	2	2	2
4	1	2	2
5	1	1	2
6	1	2	1
7	2	3	2
8	1	2	2
9	3	4	2
10	1	2	2

Close 9 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	3	3	1
2	2	3	2
3	3	2	2
4	2	2	1
5	2	3	2
6	2	2	2
7	3	3	2
8	3	3	2
9	3	3	2
10	3	3	2

Away 12 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	3	4	3
2	3	4	2
3	2	3	2
4	3	3	2
5	3	4	3
6	2	3	1
7	4	4	2
8	3	3	2
9	3	4	2
10	3	4	3

Close 12 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	1	4	4
2	2	2	3
3	3	3	3
4	4	3	3
5	5	3	4
6	6	3	1
7	7	4	4
8	8	3	4
9	9	3	4
0	10	3	3

Away 15 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	3	4	1
2	2	4	2
3	3	4	2
4	2	4	1
5	3	4	3
6	1	3	2
7	4	4	2
8	3	4	2
9	3	4	2
10	4	4	3

Close 15 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	4	4	1
2	3	4	2
3	3	4	2
4	3	3	3
5	3	3	2
6	3	3	2
7	4	4	3
8	3	4	2
9	3	4	2
10	4	4	2

Away 18 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	3	4	1
2	3	4	2
3	3	4	2
4	2	4	1
5	3	4	3
6	2	3	1
7	3	4	2
8	3	4	2
9	3	4	3
10	3	4	2

Close 18 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	4	4	2
2	4	4	3
3	3	4	3
4	3	4	3
5	3	4	3
6	3	4	3
7	4	4	3
8	3	4	2
9	3	4	3
10	4	4	3

Away 21 says	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	3	4	1
2	3	4	2
3	3	4	2
4	3	4	1
5	3	4	2
6	3	4	2
7	4	4	2
8	3	4	2
9	3	4	2
10	3	4	2

Close 21 days	ChemFil Superior	Type I Collagen 0.01%+ChemFil	Type I Collagen 0.1%+ChemFil
1	4	4	2
2	4	4	2
3	3	4	2
4	3	4	2
5	3	4	2
6	2	4	3
7	4	4	3
8	3	4	2
9	4	4	3
10	4	4	2

Appendix F- The raw data obtained from ranking the images of oral mucosa fibroblast close and away from unmodified and bio-modified ChemFil superior with different concentration of RGD for 21 days by ten observers.

AWAY 24hr	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	1	1	1
2	2	2	2
3	1	2	3
4	2	3	3
5	2	2	3
6	1	2	2
7	3	3	3
8	2	2	2
9	3	3	2
10	2	2	2

close 24hr	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	1	2	2
2	2	2	2
3	1	3	3
4	2	3	3
5	2	3	3
6	2	3	2
7	3	4	2
8	2	2	2
9	2	3	2
10	2	3	3

AWAY 3 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	1	1	1
2	2	2	2
3	1	2	2
4	2	2	2
5	2	2	2
6	1	2	1
7	2	3	2
8	2	2	2
9	2	3	2
10	2	2	2

close 3days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	1	1	1
2	2	2	2
3	1	3	2
4	2	2	2
5	2	2	2
6	1	1	1
7	3	3	3
8	2	2	2
9	2	3	2
10	2	2	2

AWAY 6 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	1	1	1
2	2	2	2
3	2	2	2
4	2	2	2
5	2	3	2
6	1	2	1
7	3	3	2
8	2	2	2
9	2	2	3
10	1	2	2

close 6days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	2	2	2
2	2	2	1
3	2	3	2
4	2	2	2
5	2	3	3
6	2	2	2
7	3	3	3
8	2	2	2
9	3	3	3
10	3	3	3

AWAY 9 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	1	1	1
2	2	3	2
3	2	2	2
4	2	3	1
5	1	1	2
6	1	2	2
7	3	1	3
8	2	2	2
9	3	2	2
10	1	1	2

close 9 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	3	1	2
2	3	3	3
3	2	2	2
4	2	2	2
5	3	2	3
6	2	2	2
7	3	2	2
8	2	2	2
9	3	3	3
10	3	4	2

AWAY 12 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil	close 12 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	2	2	1	1	3	4	1
2	3	3	1	2	4	4	3
3	3	2	2	3	2	3	2
4	3	1	1	4	3	2	2
5	3	2	3	5	3	3	2
6	2	2	1	6	3	3	2
7	4	2	1	7	4	4	3
8	3	2	1	8	3	3	2
9	3	3	2	9	3	3	3
10	3	2	1	10	4	4	2

AWAY 15 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil	close 15 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	2	2	1	1	4	3	3
2	3	3	1	2	4	4	3
3	3	2	2	3	3	3	2
4	2	2	1	4	3	2	2
5	3	2	1	5	4	3	2
6	2	2	1	6	3	4	1
7	3	3	2	7	4	3	3
8	2	2	1	8	3	3	2
9	3	3	2	9	3	3	3
10	3	3	1	10	4	4	3

AWAY 18 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil	close 18 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	3	3	3	1	3	4	1
2	4	4	4	2	4	4	2
3	3	3	2	3	3	4	2
4	3	3	2	4	3	4	2
5	3	3	3	5	3	4	2
6	2	2	2	6	3	4	1
7	4	3	3	7	3	4	3
8	3	2	2	8	3	2	2
9	4	4	3	9	4	4	2
10	4	4	3	10	4	4	2

AWAY 21 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil	close 21 days	ChemFil Superior	RGD 1 mg/ml + ChemFil	RGD 5mg/ml + ChemFil
1	4	4	3	1	4	4	1
2	4	4	3	2	4	4	3
3	3	3	3	3	3	4	2
4	3	3	2	4	3	4	2
5	3	3	3	5	4	4	2
6	2	2	2	6	3	3	1
7	4	4	3	7	4	4	3
8	3	2	2	8	3	2	2
9	3	4	2	9	4	4	3
10	4	4	3	10	4	4	3

Appendix G- The raw data obtained from ranking the images of oral mucosa fibroblast close and away from unmodified and bio-modified ChemFil superior with different concentration of Emdogain for 21 days by ten observers.

AWAY 24hr	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml	close 24hr	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml
1	1	2	1	1	1	1	1
2	2	2	2	2	2	3	3
3	1	2	2	3	1	2	2
4	2	2	2	4	3	3	3
5	2	3	2	5	3	3	2
6	1	2	1	6	2	2	2
7	2	3	2	7	3	3	3
8	2	2	2	8	2	2	2
9	2	3	3	9	2	2	2
10	2	2	2	10	2	2	2

AWAY 3 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml	close 3 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml
1	1	1	1	1	2	2	2
2	2	2	2	2	2	2	2
3	1	2	2	3	2	2	2
4	2	2	2	4	3	3	3
5	2	2	2	5	3	3	3
6	1	1	2	6	3	2	2
7	2	2	3	7	3	3	3
8	2	2	2	8	2	2	2
9	2	3	2	9	2	2	2
10	2	2	2	10	3	3	3

AWAY 6 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml	close 6 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml
1	1	1	1	1	2	3	3
2	2	2	3	2	2	3	3
3	2	2	2	3	2	3	3
4	2	2	2	4	3	4	4
5	1	2	1	5	2	4	3
6	1	1	1	6	3	3	3
7	2	2	1	7	3	4	4
8	2	2	2	8	2	2	3
9	2	2	2	9	3	3	3
10	1	2	2	10	3	3	4

AWAY 9 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml	close9 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml
1	1	1	1	1	3	2	2
2	2	2	2	2	3	3	3
3	2	2	2	3	2	3	2
4	2	2	2	4	3	2	1
5	1	2	1	5	3	3	2
6	1	2	2	6	3	2	3
7	2	2	1	7	3	3	3
8	2	2	2	8	2	2	3
9	1	2	1	9	3	3	3
10	1	2	1	10	3	3	4

AWAY 12 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml	close12 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml
1	3	2	1	1	4	3	3
2	4	3	2	2	4	3	3
3	3	2	2	3	3	3	2
4	3	2	1	4	4	2	2
5	3	3	1	5	3	3	2
6	2	2	1	6	4	3	3
7	4	3	1	7	4	3	3
8	3	2	1	8	3	2	2
9	2	2	1	9	4	3	3
10	3	3	1	10	3	4	3

AWAY 15 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml	close 15 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml
1	3	2	1	1	4	3	3
2	3	3	2	2	4	4	3
3	3	2	2	3	3	3	3
4	2	2	1	4	4	4	3
5	3	3	2	5	4	3	4
6	2	2	1	6	4	3	3
7	3	2	1	7	4	3	3
8	3	2	1	8	3	2	3
9	3	3	1	9	4	3	3
10	3	3	1	10	4	3	3

AWAY 18 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml	close 18 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml
1	3	2	2	1	4	4	3
2	3	3	2	2	4	4	3
3	3	3	2	3	3	3	3
4	2	2	1	4	3	3	2
5	3	2	2	5	4	3	2
6	3	2	1	6	3	3	2
7	4	2	1	7	4	4	3
8	3	2	1	8	3	3	2
9	3	3	2	9	3	3	3
10	3	3	2	10	4	3	3

AWAY 21 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml	close 21 days	ChemFil Superior	Emdogain 4mg/ml	Emdogain 8mg/ml
1	3	2	2	1	4	3	3
2	4	4	2	2	4	4	3
3	3	2	2	3	4	3	2
4	3	2	1	4	3	2	3
5	4	2	1	5	4	2	3
6	3	2	1	6	4	2	1
7	4	3	1	7	4	2	3
8	3	2	1	8	3	2	2
9	3	3	2	9	3	3	3
10	4	3	1	10	4	3	2

Appendix H- The raw data obtained from ranking the images of oral mucosa fibroblast close and away from unmodified and bio-modified ChemFil superior with different with Bio-additives for 21 days by ten observers.

AWAY 24hrs	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	1	1	1
2	2	2	2
3	2	1	1
4	2	3	3
5	2	2	2
6	1	2	1
7	3	3	3
8	2	2	2
9	2	2	2
10	2	2	2

close 24hrs	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	2	2	1
2	2	2	3
3	1	2	2
4	3	3	3
5	3	3	3
6	2	2	2
7	3	3	3
8	2	2	2
9	2	2	2
10	3	2	2

AWAY 3 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	1	1	1
2	2	2	2
3	2	1	1
4	3	3	3
5	2	2	3
6	1	2	1
7	3	2	2
8	2	2	2
9	2	2	2
10	2	2	2

close 3 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	1	2	2
2	2	2	3
3	2	2	2
4	3	3	3
5	2	2	3
6	2	3	2
7	3	3	3
8	2	2	2
9	2	2	3
10	2	2	3

AWAY 6 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	1	1	1
2	2	2	3
3	2	2	2
4	3	3	2
5	3	2	2
6	2	2	1
7	3	3	2
8	2	2	2
9	1	1	1
10	1	1	1

close 6 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	1	2	4
2	3	3	4
3	2	2	3
4	3	3	4
5	2	2	4
6	2	3	3
7	3	3	4
8	2	2	3
9	2	2	3
10	2	2	4

AWAY 9 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	1	1	1
2	2	2	2
3	2	2	2
4	2	2	2
5	2	3	2
6	1	2	1
7	2	3	2
8	2	2	1
9	1	1	1
10	2	1	1

close 9 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	1	3	3
2	3	3	3
3	2	2	2
4	2	2	3
5	2	3	2
6	2	3	2
7	3	3	3
8	2	2	2
9	2	3	3
10	2	3	3

AWAY12 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml	close 12 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	2	1	1	1	2	2	2
2	2	1	2	2	3	3	3
3	2	2	2	3	2	2	2
4	2	2	2	4	2	2	3
5	2	1	1	5	1	2	2
6	2	1	1	6	2	2	2
7	2	3	2	7	2	3	3
8	2	1	1	8	2	2	2
9	2	1	1	9	2	2	3
10	2	1	1	10	2	2	3

AWAY15 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml	close 15 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	1	1	1	1	2	2	3
2	2	1	2	2	3	2	3
3	2	2	2	3	2	3	2
4	2	2	2	4	3	2	3
5	1	1	1	5	2	3	4
6	1	1	1	6	3	3	3
7	3	1	1	7	3	3	4
8	1	1	1	8	2	2	3
9	2	2	2	9	1	3	3
10	2	1	1	10	2	2	3

AWAY18 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml	close 18 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	1	3	1	1	3	2	3
2	2	3	3	2	3	2	3
3	2	3	2	3	3	2	2
4	1	3	1	4	3	2	2
5	1	2	1	5	2	2	2
6	1	2	1	6	3	2	3
7	1	3	1	7	4	3	3
8	2	2	1	8	2	2	2
9	2	2	2	9	3	3	3
10	2	3	2	10	3	2	3

AWAY21 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml	close 21 days	Collagen 0.1%+ChemFil	RGD 5mg/ml + ChemFil	Emdogain 8mg/ml
1	2	3	1	1	2	2	3
2	1	3	2	2	2	3	3
3	2	3	2	3	3	3	3
4	1	3	1	4	2	2	2
5	2	3	1	5	2	2	2
6	1	2	1	6	2	2	2
7	1	4	4	7	3	3	3
8	2	2	1	8	2	2	2
9	2	3	2	9	3	3	3
10	2	3	2	10	3	2	3